THE URINARY EXCRETION OF RIBOFLAVIN BY COLLEGE WOMEN [1]

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TWO FIGURES

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INTRODUCTION

Parsons ('44) and Copping ('45) have reviewed the literature concerned with the metabolism of riboflavin. Except in conditions of extreme deficiency, in which case specific symptoms may be present (Goldsmith, '43), the means of assessing riboflavin nutrition depend upon the urinary excretion of the vitamin. Apparently urinary losses parallel intakes closely until a point is reached at which higher intakes result in larger quantitative excretion but little increase in percentage excretion. The urinary excretion of riboflavin may also depend upon the nitrogen content of the diet (Sarett and Perlzweig, '43). Since there is no recognized depot for riboflavin storage in the body, it is possible that increased destruction occurs at the higher intakes. An adequate or "optimal" intake of riboflavin might be defined as the upper limit of intake at which there is economical utilization of the substance. This study was planned to investigate the use of riboflavin by young women in the higher ranges of intake at which the body retains an economical proportion of the ingested nutrient.

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SERIES A

This consisted of a study of the urinary excretion of riboflavin by twenty college women on self-selected diets.

Experimental plan

The urinary excretion of riboflavin of twenty college women was studied for a 4-day period. These women were living in their usual college residences and the diet was self-selected. Twenty-four-hour urine samples were collected for the first 3 days. On the fourth morning a 1-hour fasting sample was obtained. At this time the subject was given an oral test dose containing 3 mg of riboflavin and 3 mg of thiamine hydrochloride [3] and a light breakfast. Urine was collected for the following 4-hour period and a separate collection of urine was made for the next 19 hours.

The riboflavin content of the urine was determined by the adsorption procedure of Conner and Straub ('41) as modified by Keys ('44). The degree of fluorescence was measured in a photo-electric fluorescence meter.[4] The instrument was standardized against a solution containing 0.1 μg of riboflavin per ml.

Results and discussion

The urinary excretions of riboflavin for these subjects are given in table 1. The range of urinary riboflavin for the 3-day period on the self-selected diet was from 0.06 to 0.92 mg in 24 hours. Two students excreted less than 0.10 mg per 24 hours. No riboflavin was demonstrable in the 1-hour fasting sample excreted by the subject for whom the lowest value, 0.06 mg, was obtained in the 3 days on the non-restricted diet. The urinary values following the test dose were correspondingly low for this subject. Fifteen per cent of the supplement was excreted in 4 hours and 20% in 24 hours. The percentage excretion of the test dose excreted in 24 hours was calculated

[3] Acknowledgment is made to Hoffmann-LaRoche, Inc., for the supply of riboflavin and thiamine hydrochloride.

[4] Lumetron, Will Corporation.

from the difference between the urinary value obtained after the administration of the dose and the average excretions for 3 days on the self-selected diet.

The group as a whole excreted from 9 to 57% of the 3-mg test dose in the 4 hours following its administration. In 24 hours after the test dose, 4 of the twenty subjects excreted less than 20% and ten of the subjects excreted more than 30% of the added supplement. The highest excretion was 68%.

TABLE 1

Urinary riboflavin excretion of twenty college women.

CONDITIONS	URINARY EXCRETION OF RIBOFLAVIN	
No. of cases	2	18
Average, 3 days on non-restricted diet		
mean, mg per day	0.08	0.49
range, mg per day	0.06–0.10	0.16 –0.92
S.D.,[1] mg		0.04
1-hour fasting		
mean, mg per hr.	0.004	0.02
range, mg per hr.	0–0.008	0.001–0.04
S.D., mg		0.005
4 hours after oral test dose		
Mean, mg per 4 hrs.	0.50	0.82
range, mg per 4 hrs.	0.46–0.54	0.26 –1.72
S.D., mg		0.10
24 hours after oral test dose		
mean, mg per 24 hrs.	0.76	1.47
range, mg per 24 hrs.	0.67–0.84	0.52 –2.80
S.D., mg		0.13
Correlation coefficients		
Average excretion for 3 days on non-restricted diet with:		
1-hr. fasting excretion	— 0.602[2]	
4-hr. excretion after oral test dose	— 0.570[2]	
24-hr. excretion after oral test dose	— 0.736[2]	
1-hr. fasting excretion with:		
4-hr. excretion after oral test dose	— 0.338	
24-hr. excretion after oral test dose	— 0.592[2]	

[1] Standard deviation of the mean.

[2] Less than one chance in 100 of a fortuitous result.

The average urinary excretion for the 3 days on the self-selected diet was significantly related to (a) the 1-hour fasting excretion, (b) the 4-hour excretion after the oral test dose, and (c) the 24-hour excretion after the test dose. There was also a significant relationship between the 1-hour fasting excretion and the 24-hour excretion after the test dose. The correlation coefficient obtained between the 1-hour fasting test and the 4-hour excretion after the test dose did not possess significance. It is possible that irregular absorption of riboflavin occurs following the oral dosage. Our observations, with this exception, support the conclusion of Oldham and others ('44), that the 1-hour fasting excretion, the 4-hour, and the 24-hour return of a test dose reflect equally well the nutritional status with respect to riboflavin. However the difficulty in determining the small amounts of riboflavin in the 1-hour fasting excretion supports the criticism by Hagedorn et al. ('45) of this technique. For the purpose of the controlled dietary study in Series B, the 24-hour urinary excretion after a test dose was selected for evaluation of the riboflavin status of the individual.

SERIES B

This series consisted of a study of the urinary excretion of riboflavin by fourteen college women on controlled intakes.

Experimental plan

Fourteen college women acted as subjects for this phase of the study. The subjects were in good physical health throughout the experiment as established by physical examinations. The age, height, and weight of the subjects are given in table 2.

The urinary excretion of riboflavin by the subject on her customary, self-selected diet was studied for 6 days. For 6 succeeding days the customary diet of the subject was supplemented daily with 3 mg of riboflavin and 3 mg of thiamine hydrochloride and the urinary excretion of riboflavin was determined. For the following 12-day period the subject re-

ceived the experimental diet only and for two additional periods of 12 days each she received the experimental diet supplemented with riboflavin. On the day following each of these periods the subject was given the experimental diet and a test dose of 3 mg of riboflavin. For 3 days preceding the second and third periods on the experimental diet the subjects returned to a self-selected diet supplemented with 3 mg of riboflavin and 3 mg of thiamine so that a high tissue storage of vitamin was present at the beginning of each experimental period.

TABLE 2

Physical characteristics of subjects.

SUBJECT	HEIGHT	WEIGHT	AGE	SUBJECT	HEIGHT	WEIGHT	AGE
		kg				*kg*	
BN	5'9"	66.3	23	BY	5'5"	53.0	22
JF	5'6"	68.2	24	GB	5'4"	65.7	20
MJ	5'4"	65.3	22	VB	5'5"	63.1	27
PK	5'7"	60.4	22	LK	5'4"	54.1	28
EM	5'	52.6	24	AC	5'4"	54.3	27
BS	5'	50.5	21	BM	5'2"	45.5	32
EJ	5'4"	54.6	22	RI	5'	54.6	28

Experimental diet. The low riboflavin diet was planned so that menus were repeated every 3 days (table 3). The diet provided from 2100 to 2300 cal. daily. The calorie requirement of each subject was estimated on the basis of her activity, size and food habits. Adjustments in the calorie value of the diet were made by variations in the amounts of riboflavin low foods such as butter, sugar and bread prepared from non-enriched flour. Each serving of food was weighed and it was assumed that the calorie value of the diet was constant for each subject throughout the period of study. The weights of the subjects remained uniform throughout the experiment. The riboflavin content of the milk in the diet was partially destroyed by exposure of the milk to ultraviolet light for 30 minutes with continuous stirring. This treatment produced an undesirable change in flavor but all of the subjects were able to accustom

themselves to the flavor. Daily supplements to the diet were 0.5 gm of tri-calcium phosphate and one-half teaspoon of cod-liver oil. Analyses of the dietary intakes and urinary excretions of thiamine by the subjects will be reported elsewhere; the dietary intake of thiamine was adequate to maintain a constant urinary excretion of thiamine.

TABLE 3

Experimental diet. Total foods included in 3 days.[1]

FOOD	WEIGHT	FOOD	WEIGHT	FOOD	WEIGHT
	gm		*gm*		*gm*
Farina, cooked	405	Corn, whole kernel	70	Apple sauce	126
Bread, white non-enriched	300	Green string beans	67	Pears	100
Saltines	12	Peas	70	Apple, cooked	132
Spaghetti, cooked	120	Cabbage, shredded	43	Grapejuice	100
Rice, cooked	100	Carrot strips	20	Dates, stoned	25
Milk	732	Celery	20	Sugar cookies	84
Butter	156	Split pea soup	196	Brownies	38
Cream	60	Tomato juice	100	Apple sauce cake	50
Beef, ground, cooked	264	Grapefruit juice	360	Sugar	52
Bacon, cooked	16	Fruit cocktail	200	Jelly	144
Potato	360	Peaches	170	Mayonnaise	14
				Gelatin	24

[1] By analysis: thiamine, 0.62 mg/24 hrs.; niacin, 9 mg/24 hrs.; and riboflavin, 0.79 mg/24 hrs.

Methods. The riboflavin content of the diet was analyzed daily by a modification of the method of Conner and Straub ('41). An aliquot which represented one-fifth of the amount served was taken of each food included in the day's diet exclusive of butter and sugar. The food was stored under 2% acetic acid and this concentration of acid was used for rinsing dishes. Combined aliquots were blended and samples were taken for analyses.

Twenty-four-hour collections of urine were made in amber glass bottles which contained glacial acetic acid. Riboflavin analyses were made on combined aliquots of three successive 24-hour collections which were held in a household electric

refrigerator until the 3-day collections were complete. In the early period of the work, the riboflavin in the urine was determined by a direct fluorometric procedure (Sure and Ford, '42) but this method was found unsatisfactory for urines of low concentration. For samples of urine containing 1.1 mg of riboflavin or more, values obtained by the direct procedure and the adsorption procedure compared closely. The adsorption procedure described under series A was used for all urinary data reported except as indicated in the tables.

Results and discussion

In the preliminary periods, when the food intake was not restricted, the average urinary excretion of riboflavin by the subjects was from 0.14 to 1.07 mg (table 4). This range was similar to that for the subjects in series A and to the range of urinary values reported by Sebrell et al. ('41).

When the self-chosen diet was supplemented with 3 mg of riboflavin daily for 6 days, the excretion of urinary riboflavin was from 31 to 82% higher than for the preceding 6 days when no supplement was given. Since the diets in this period were not controlled, the variations in the per cent of the added

TABLE 4

Urinary excretion of riboflavin by fourteen subjects on self-selected diets and after supplementation of diet with 3 mg riboflavin daily.

CONDITIONS	URINARY EXCRETION OF RIBOFLAVIN	
	Mean	Range
	mg/24 hrs.	*mg/24 hrs.*
Self-chosen diet		
First 3 days	0.56 ± 0.069 [1]	0.12–0.99
Second 3 days	0.62 ± 0.075	0.17–1.15
Self-chosen diet supplemented with 3 mg riboflavin		
First 3 days	1.96 ± 0.123	1.24–2.96
Second 3 days	2.30 ± 0.176	1.41–3.79
Per cent of supplement excreted in urine, 6 days	52 ± 3.7	31–82

[1] Standard deviation of the mean.

riboflavin excreted by the subjects may have represented a change in basal excretion resulting from an increased or lowered amount of riboflavin in the diet. The values for urinary riboflavin during the 6-day supplementation period ranged from 1.24 to 3.79 mg per 24 hours. These values and the estimated percentage excretion of the added riboflavin indicated that the subjects were in a state of riboflavin nutrition which approached saturation, as it may be defined from urinary excretion of the vitamin.

TABLE 5

Average urinary excretion of riboflavin by college women on controlled intakes.

NUMBER OF SUBJECTS	DIETARY INTAKE OF RIBOFLAVIN	URINARY EXCRETION OF RIBOFLAVIN			URINARY EXCRETION AFTER 3 MG TEST DOSE FOLLOWING DIETARY PERIOD		
		Mean	Range	Per cent of intake	Mean	Range	Per cent of intake [1]
	mg/24 hrs.	*mg/24 hrs.*	*mg/24 hrs.*	*%*	*mg/24 hrs.*	*mg/24 hrs.*	*%*
9	0.79	0.07	0.04 – 0.10	9	0.84	0.65 – 1.10	22
3	1.04	0.16	0.14 – 0.20	15	1.18	1.03 – 1.38	30
9	1.26	0.13	0.07 – 0.25	10	1.04	0.62 – 1.45	27
9	1.62	0.32	0.17 – 0.44	20	1.23	1.02 – 1.43	31
4	2.23	1.18 [2]	1.11 [2] – 1.29 [2]	53	2.08 [2]	2.00 [2] – 2.17 [2]	55
4	2.73	1.31 [2]	1.27 [2] – 1.35 [2]	48	2.12 [2]	1.95 [2] – 2.26 [2]	56

[1] $\frac{\text{Urinary excretion of riboflavin}}{\text{Riboflavin content of basal diet} + 3 \text{ mg riboflavin}} \times 100.$

[2] Values determined by direct fluorometric procedure.

The average urinary excretion of riboflavin at each of the different riboflavin intakes is given in table 5. The values for riboflavin intake represent the average intake for each 12-day period. The excretion during the first 3 days of each period was influenced by the preceding days when a riboflavin supplement was given. By the end of 6 days the urinary riboflavin became essentially constant. Therefore, riboflavin excretions for each period were averaged from values for the last 3 days of that period. The mean excretion for nine subjects at an intake of 0.79 mg was 0.07 mg riboflavin per 24 hours, or 9% of the intake. Urinary riboflavin was increased to a mean

value of 0.13 mg per 24 hours when the intake was increased to 1.26 mg although the percentage excretion of the vitamin remained approximately 10% of the intake.

Both the absolute values for urinary riboflavin, mean, 0.32 mg per 24 hours, and the percentage excretion, 20%, were higher at an intake of 1.62 mg than at the lower intakes. A sharp rise in excretion occurred when the riboflavin in the diet was increased to 2.23 mg per day. The mean excretion was 53% of the intake. Gardner et al. ('43), reported that for riboflavin intakes of 7.0 mg, an average of 3.4 mg or 49% of the dietary riboflavin was excreted in the urine. A similar percentage excretion of riboflavin was reported by Keys ('44) for young men who received 11.2 mg per day. For our subjects an intake of 2.23 mg per day apparently resulted in as high a proportionate excretion of riboflavin as would occur on greatly increased intakes.

The validity of the 24-hour excretion of riboflavin following an oral test dose of 3 mg as a measure of riboflavin nutrition was discussed in series A. Again for the group of subjects on controlled dietary intake, the 24-hour excretion of riboflavin following the test dose was dependent upon the previous dietary intake of riboflavin of the individual. Of the fourteen subjects studied, four subjects (BS, BY, GB, VB) showed an increase in urinary riboflavin after the test dose following an intake of 1.2 mg riboflavin and for these subjects the excretion of test dose after an intake of 1.6 mg was approximately the same as after an intake of 1.2 mg. The urinary excretion of riboflavin for successive 3-day periods for each dietary intake and the 24-hour excretion of riboflavin after each test dose were averaged for these subjects and the average values are shown in figure 1. For four subjects (JF, MJ, BN, PK) the increase in urinary excretion of riboflavin after the test dose occurred following a dietary intake of 1.6 mg. For these subjects the upper limit of intake which appeared to maintain tissue stores was not determined but an intake of 1.6 mg seemed to approach or exceed this point. For the subjects AC, RI and LK, approximate tissue satura-

tion apparently occurred between intakes of 1.0 and 2.2 mg per day.

A scatter diagram (fig. 2) was prepared from the data from the study reported here and from values for women of the urinary excretion of riboflavin at known intakes which have been reported in the literature. These include the data reported by Strong et al. ('41), Williams et al. ('43), Davis ('45), Sebrell et al. ('41) and Gardner et al. ('43). The distri-

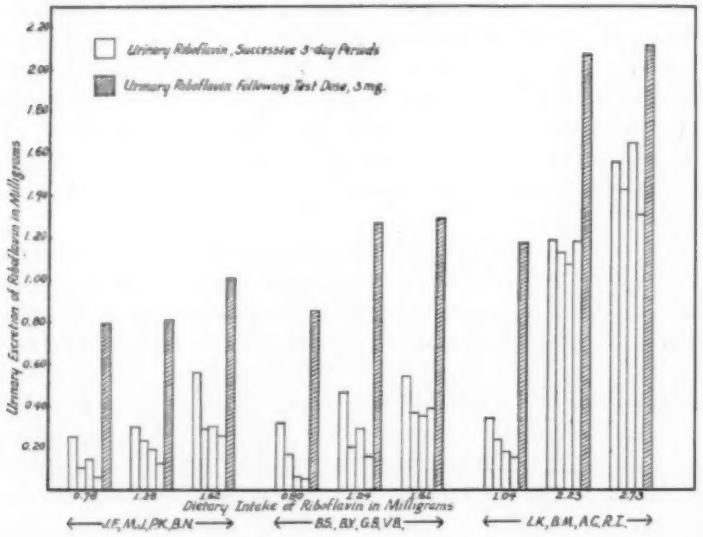

Fig. 1 Excretion of riboflavin of twelve subjects on three dietary intakes and following a 3-mg test dose of riboflavin.

bution of values indicated that a gradual increase in urinary excretion of riboflavin occurred with increasing intakes from 0.50 mg to intakes of from 1.3 to 2.0 mg riboflavin per day. For intakes above this range, a sharp increase in excretion occurred with increased intakes. Using the predicting equation, $y = a + bx$, regression lines were plotted from values from this study, from values from the literature, and from the combined values. For each group of data, one regression

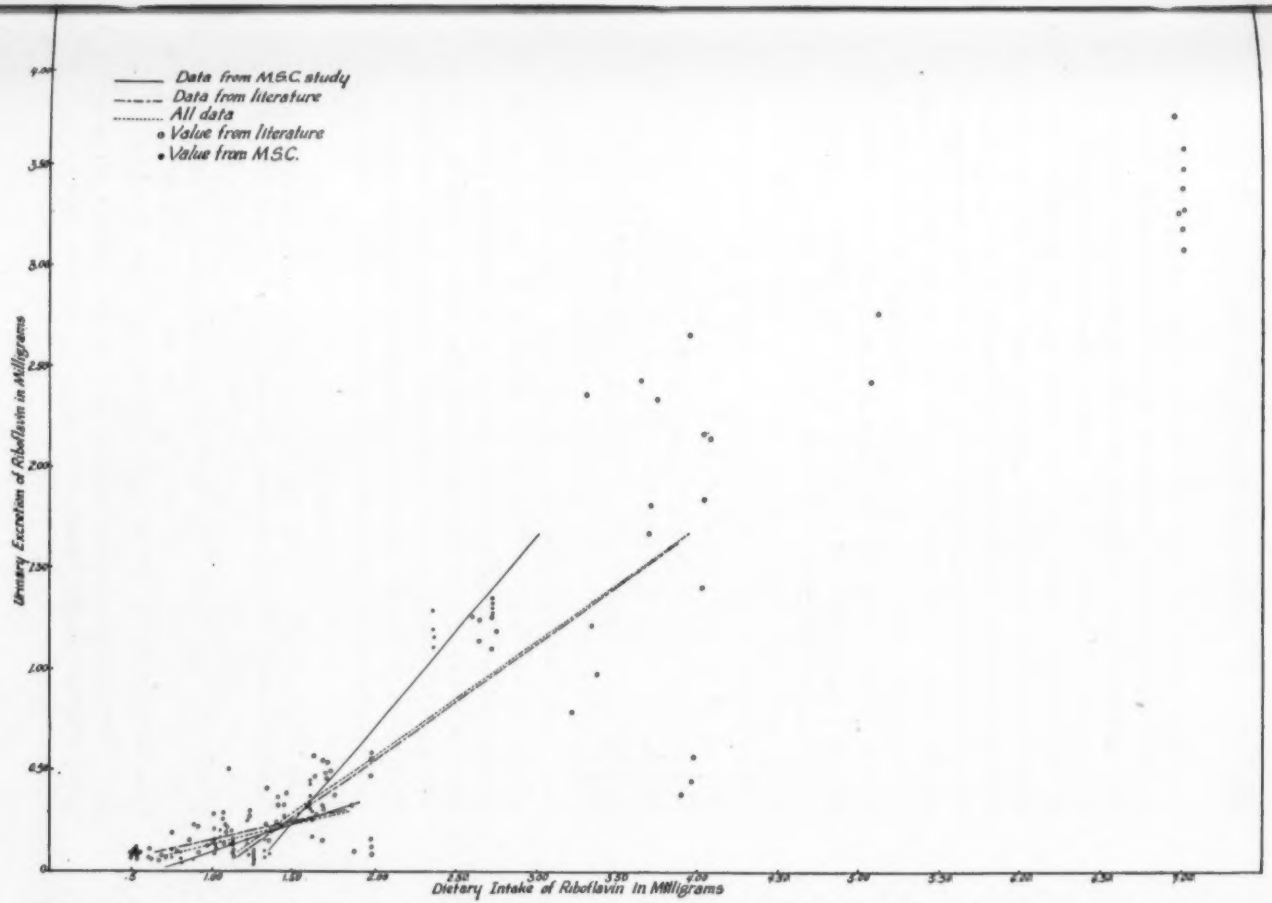

Fig. 2 Urinary excretions of riboflavin at intakes ranging from 0.50 to 7.0 mg: data on women selected from the literature combined with data from this study.

line was plotted for urinary riboflavin at intakes from 0.50 to 2.0 mg and another line from values at intakes of 1.3 mg and above. These limits were selected from the apparent distribution of values on the scatter diagram. The "t" test for significance was applied to the slope of the lines. The slope of the line of values obtained in our study did not differ significantly from the slope of the corresponding line obtained for urinary values reported in the literature. The "t" values for the slopes of the lines predicted from values below 2.0 mg was 1.6; for 1.3 mg and above, the "t" value was 1.08. There was, however, a significant difference between the slope of the lines for values at intakes of 0.50 to 2.0 mg riboflavin per day as compared with the slope of the lines for values at intakes of 1.3 mg riboflavin and above. The "t" value was 3.48.

The points of intersection of lines in the graph lie between intakes of 1.3 and 1.5 mg riboflavin per day. At this area of intake there was an abrupt change in the retention of dietary riboflavin. When the riboflavin content of the diet exceeded an intake of 1.5 mg per day, a rapid rise in excretion of urinary riboflavin occurred with increasing intakes. Under the defined conditions of this experiment and under the varying conditions of study of riboflavin excretion of women as reported in the literature, it would appear that from 1.3 to 1.5 mg riboflavin in the diet represents the upper limit of economical utilization of the vitamin for women with an intake of 2100 to 2300 cal. We have interpreted these findings to indicate that tissue supplies will be adequate under these conditions.

In their study of the riboflavin content of the average American diet, Cheldelin and Williams ('43) reported that diets providing 2500 cal. could be expected to contain 1.4 mg riboflavin and that with enrichment of cereal, the riboflavin could be increased to 1.6 mg. Thus the selection of a sufficient amount of total food for maintenance and the provision of the calcium and protein requirement would supply an adequate amount of riboflavin.

SUMMARY

The urinary excretion of riboflavin by college women was studied on non-restricted diets, after an oral test dose, and on controlled riboflavin intakes.

The urinary excretions for twenty women on self-selected diets ranged from 0.06 to 0.92 mg in 24 hours. The 1-hour fasting excretion, the 4-hour and the 24-hour excretions after an oral test dose of 3 mg were significantly related with the average daily urinary riboflavin excretion for 3 days preceding the test dose administration.

From three to nine subjects were studied at each of the following riboflavin intakes: 0.79, 1.04, 1.26, 1.62, 2.23 and 2.72 mg daily. The average urinary riboflavin for the last 3 days of each period of controlled diet were as follows: 0.07, 0.16, 0.13, 0.32, 1.18 and 1.31 mg per 24 hours. The percentage of a 3-mg test dose excreted in 24 hours following the periods of controlled intake was, respectively, 22, 30, 27, 31, 55 and 56.

A comparison of the data from this study and the data reported in the literature indicated that 1.3 to 1.5 mg riboflavin per day were adequate for women with a requirement of 2100 to 2300 cal.

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We wish to express our appreciation for the cooperation of the college women who acted as subjects and to Mrs. Annanell Jubb for her technical assistance. We are grateful to Dr. W. D. Baten for his advice and help in the statistical analysis of the data.

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BIOLOGICAL VALUE OF PROTEINS IN RELATION TO THE ESSENTIAL AMINO ACIDS WHICH THEY CONTAIN

IV. THE ANALYSIS OF FIFTEEN PROTEIN FOODS FOR THE TEN ESSENTIALS [1]

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In the third paper of this series (Murlin, Edwards, Fried and Szymanski, '46) reference was made to the analyses upon which were based the mixtures of essential amino acids used in that study in comparison with the proteins which the mixtures simulated. The present report deals with the methods as checked and verified by recovery of the pure synthetic products of Merck and Co. in the case of each acid, and the final results obtained for the proteins selected for the comparison. It must be admitted here again that some of the mixtures compared for biological values with the proteins were not satisfactory because some of the methods used for analysis up to the time the mixtures were compounded had not been adequately checked. Consequently the emphasis in paper III was placed only on the use of mixtures in the later comparisons when the analyses had been more thoroughly tested.

In the description of the methods of assay of the individual acids will be found statements of the average percentage

[1] This work was supported by generous grants from the Nutrition Foundation.

recovery of the pure substances found. Correction has been made in the tables for these recovery figures.

It was confirmed also that lysine, the most vulnerable of these amino acids to destruction by acid hydrolysis (except, of course, tryptophane for which an alkaline hydrolysis always was used) was not destroyed under the conditions which prevailed in the hydrolysis as carried out in this study.

HYDROLYSIS OF FOOD MATERIALS

Treatment of food samples

Meats and fish, freed of extraneous tissue, and egg were treated with dry acetone and ether to remove fat. For example, 150 gm of ground meat were stirred with six volumes of acetone in a Waring blender for 3 minutes. The mixture was allowed to stand for 1 hour with occasional shaking. The solution then was filtered and the residue allowed to dry under suction. As soon as it was dry to the touch the powder was extracted with six volumes of ether in a similar fashion. This process removed the water and most of the fat.

The farinaceous foods, the yeasts and legumes were treated with acetone in the same manner as the animal foods, but for extraction of the acetone dried material the Soxhlet was used and extraction of fat continued over-night. The loss of nitrogen into the acetone from these materials was usually less than 10 mg per 100 gm; but from peanut meal it was 16.7 mg and from Navy bean meal, prepared in the laboratory, it was 112 mg per 100 gm. The loss into anhydrous ether was never more than 3 mg per 100 gm.

One gm of dry protein was placed in a test tube 20 mm by 200 mm. Then 30 ml of 20% HCl was added to the contents in the tube. A 25 ml Erlenmeyer flask was inverted over the test tube. The test tube and contents were then placed in an oil-bath at 120–130°C. for 24 hours. The level of the solution in the test tube was always kept at the same level as that of

TABLE 1

The decarboxylase method compared with the microbiological method for lysine in the entire series of fifteen proteins.

	AS PERCENTAGES			
	A. In protein (16% N)		B. In whole food	
	Microbiological	Decarboxylase	Microbiological	Decarboxylase
Whole egg	8.2	7.8	1.00	0.96
Beef (lean round)	8.1	8.2	1.22	1.23
Horse meat (tough, stringy)	8.5	7.6	1.64	1.47
Halibut (large steaks)	9.7	9.5	1.95	1.90
Haddock (fresh, boneless)	9.4	9.6	1.82	1.86
Soybean flour [1] (fat extracted)	6.0	5.9	2.98	2.97
Navy beans, dry	6.7	6.4	1.49	1.43
Brewer's yeast [2] (blend of three)	6.6	7.4	2.90	3.24
Torula yeast [3]	5.7	6.8	2.52	3.00
"Kitchen Food" [4] yeast	6.3	6.3	2.78	2.76
Corn germ [5] (defatted)	4.8	5.2	1.03	1.10
Wheat germ [5] (defatted)	5.7	5.1	2.11	1.89
Cotton seed meal [6] (defatted)	4.3	3.4	2.30	1.81
Peanut flour [7] (defatted)	3.6	3.0	2.04	1.67
Sunflower seed meal [6] (defatted)	3.1	3.0	1.50	1.45

[1] Contributed by The Glidden Co., Chicago, Ill.

[2] Contributed by The Yeast Products, The Pabst, and The Heffenreffer Companies.

[3] Torulopsis utilis, through the courtesy of Dr. Thyssen, London, Eng.

[4] Contributed by the Anheuser-Busch Co., St. Louis, Mo.

[5] Purchased from The Vio Bin Corp., Monticello, Ill.

[6] Purchased from The Oil Mill Products Co., Ft. Worth, Tex.

[7] Obtained through the courtesy of Dr. D. Breese Jones, Washington, D. C.

the oil-bath during hydrolysis. The contents were then transferred to a beaker or shallow dish and dried by warm air or by reduced pressure in a desiccator. This drying was always kept at room temperature.

Hydrolysis for arginine and histidine

Both arginine and histidine analyses as described can be run on diluted HCl hydrolysates of proteins without removing the acid. In these experiments 250 mg of dried defatted protein were placed in a test tube of convenient size. Five ml of 6 N HCl were added, a boiling chip inserted and the tube covered with a glass-bulb air condenser. The mixture was gently refluxed for 24 hours in an oil-bath at 120°C. After hydrolysis the contents of the tube were filtered and diluted to 25 ml. If this solution is again diluted 1 to 10, a sample of 1.0 ml with most proteins will give a color convenient for both the arginine and histidine colorimetric methods of analysis. Neither the final acidity (1.0 ml of 0.12 N HCl) nor the color of the hydrolysate interferes with the Sakaguchi or the Pauly reaction. Adding pure solutions and, alternatively, pure solid arginine and histidine to the food sample before hydrolysis gave recoveries of 95 to 100%.

Hydrolysis for lysine

The destruction of lysine during hydrolysis was carefully checked. When added to 20% HCl and the protein and heated to 120°C. for 24 hours 100% recovery of the amount added was obtained when the hydrolysate was dried down in the desiccator. Although not destroyed by this method of hydrolysis and drying,[2] a second heating to not higher than 40°C. can be very destructive. Care should be taken therefore in redrying a diluted sample from a hydrolysate, especially from foods with a high lysine content.

[2] A quick and satisfactory method of drying hydrolysates, not apparently widely known, is the following:

A mixture of flaked sodium hydroxide and calcium chloride (1 to 1) is used as the drying agents in the desiccator. Suction is applied by a water pump and as hydrochloric acid comes off first, the pressure remains high for several hours. When the vapor pressure of water is reached and maintained for an hour the system is closed. If the system is not closed, the drying agents pick up water from the pumps. As the drying agents may become hot, the desiccator must be cooled in a water bath. A sample can be dried by this method in about 36 to 48 hours.

INDIVIDUAL METHODS OF ANALYSIS

Arginine

The determination of this diamino acid in the catholyte, obtained as the second product of electrodialysis for all the basic forms, was carried out by means of the modification of Weber's quantitative use of the Sakaguchi reaction, introduced by Thomas, Ingalls and Luck ('39). The results were conflicting as much because of difficulties with the physical method as with the Sakaguchi reaction. It proved exceedingly

TABLE 2

Comparison of values for "leucine" group by microoxidative and microbiological procedures.

(Percentage figures on basis of 16% N).

	LEUCINE		VALINE		ISOLEUCINE	
	Oxidative	Microbiol.	Oxidative	Microbiol.	Oxidative	Microbiol.
Halibut	11.6	8.8	5.8	6.0	6.1	6.2
Haddock	12.5	8.6	4.2	5.9	3.2	6.2
Beef	13.1	7.7	3.9	5.2	5.5	5.7
Horse meat	10.9	7.8	4.5	5.3	6.5	5.1
Egg	17.8	9.7	5.2	7.2	5.1	7.0
Peanut flour	15.5	6.8	1.3	5.3	4.5	4.6
Cottonseed flour	10.4	6.0	4.6	5.1	4.7	3.9
Sunflower seed	12.0	6.7	3.1	5.6	4.5	5.1
Yeast blend	18.1	7.8	2.7	6.4	6.8	6.0
Wheat germ	8.6	6.6	3.7	6.3	4.0	3.8
Navy beans	11.1	8.2	4.4	6.0	3.6	5.8
Corn germ	10.3	7.7	4.0	5.9	3.5	4.1

troublesome with either of two forms of electrophoretic cells to secure duplicate values for any of the basic amino acids from two aliquots of the dried hydrolysates. Critical examination of the procedures for use of the Sakaguchi reaction also was disappointing because all were found to have serious disadvantages.

As is well known the basic reaction is the coupling of arginine with α-naphthol in alkaline solution, and conversion to a red compound by means of sodium hypobromite. Because excess of the hypobromite destroys the color, attempts

are made to decompose the excess by quickly adding urea solutions, or to run the analysis in the presence of urea. Careful control of the time of adding the reagents and use of varying amounts and concentrations of the hypobromite have been required. Any given procedure in our hands has proved difficult of control so as to obtain a readable color proportional to the concentration of arginine. The required improvement was a means of stabilizing the color. This was accomplished by one of us (G.R.B.) in discovering that n-butanol serves not only to extract the red compound from the aqueous mixture but also to stabilize it so that the same reading can be relied upon up to 1 hour after inception.

The reagents are essentially the same as those introduced by Weber ('30). The procedure is as follows: A 5 ml sample of the hydrolysate (or catholyte) containing 5 to 100 μg of arginine is placed in a test tube, followed by 1.0 ml of 2.5 N NaOH and finally by 1.0 ml of the α-naphthol solution (0.05% in 25% ethanol). Mixing quickly the reaction is completed within 1 minute. Now add 0.2 ml of the hypobromite solution with shaking and immediately blow [3] into the mixture 10 ml of n-butanol saturated with water. Give the tube a few seconds of vigorous shaking and allow the butanol layer to separate. Suck out the bottom aqueous layer, add 1 ml of 95% ethanol to clarify the butanol solution and read at will up to 1 hour.

The absorption is maximal at 500 mμ. Of course a blank should be run on the reagents which alone give a yellowish color, but with a selective photometer set for clearance at 500 mμ this color is largely filtered out. It has been found convenient to run the whole reaction in test tubes calibrated to fit the Coleman Universal Spectrophotometer.

Under the conditions described the red color not only is stable but is proportional to the concentration of arginine used. Samples should have a pH and buffering power such that no marked shift is produced by addition of the alkaline

[3] Blowing the butanol into the solution with a pipette produces a quicker mixing of the relatively immiscible solvents and a prompter extraction of the color.

reagents. Below pH 11.0 a series of varied color reactions is produced.

All the values given in tables 3 and 4 are derived by this Bartlett modification of the procedures hitherto used.

Histidine

The reagents used are essentially those employed by the MacPherson modification of the Pauly ('04) diazotization reaction. It was confirmed that under the conditions outlined below, low temperature manipulations, a disadvantage of previous modifications of this method, were unnecessary. The amounts of reagents used have been adjusted to get greater sensitivity and convenience for photometric analysis in the Coleman Colorimeter. The whole determination was handled in colorimeter tubes. We found it was unnecessary to wait more than 1 minute for the diazotization-histidine coupling reaction (Macpherson ('42) used 30 minutes).

The procedure is as follows: place 3.0 ml of a sample containing in this volume 2 to 40 mg of histidine in the colorimeter test tube. Add 5 ml of 1% sulfanilic acid solution followed by 0.5 ml of 5% sodium nitrite. After 1 minute the diazotization and the coupling with histidine are complete. Add 1.5 ml of the 20% sodium carbonate to develop the color. Mix well and add 4.5 ml of this 75% ethanol, which substance Macpherson found to be satisfactory for stabilizing the color. The interference of tyrosine in acid protein hydrolysates is negligible.

The color is proportional to the concentration of histidine used, and its maximal absorption occurs at 500 mμ. The whole analysis can be carried out at room temperature.

Adsorption. As an additional check against impurities which might give the Sakaguchi or the Pauly reactions, adsorption separation has been tried. The cation adsorbents Amberlite IR. 100 HAG [4] and ZeoKarb H [5] were found to

[4] Resinous Products and Chemical Co.

[5] Block and Bolling ('45, p. 42) suggest that a combination of Permutit adsorption (Dubnoff) and Macpherson's method may be the best application of the Sakaguchi reaction.

TABLE 3

Essential amino acids in fifteen food proteins expressed as percentages of N × 6.25 of the acetone-ether insoluble fraction.

	ARGININE	HISTIDINE	ISO-LEUCINE	LEUCINE	LYSINE	METHIONINE	PHENYL-ALANINE	THREONINE	TRYPTOPHANE	VALINE	% OF A-E INSOL. IN WHOLE FOOD	% N IN A-E FRACT.
1. Whole egg	9.70	3.64	7.00	9.70	7.80	3.88	6.10	4.94	1.63	7.20	15.50	12.72
2. Beef (lean, round)	10.10	4.40	5.70	7.70	8.15	4.12	3.55	5.14	1.46	5.20	16.67	14.46
3. Horse meat (tough, stringy)	8.70	4.18	5.10	7.80	7.60	6.23	3.55	4.41	0.61	5.31	22.45	13.80
4. Halibut (large steaks)	8.40	3.68	6.20	8.80	9.44	4.48	3.39		1.10	6.00	21.80	14.72
5. Haddock (fresh, boneless)	9.20	3.32	6.20	8.60	9.55	4.07	4.14	3.95	1.23	5.90	21.34	14.60
6. Soy bean flour (fat extracted)	11.00	3.60	5.70	7.60	5.93	3.59[1]	5.14	5.24	1.08[1]	5.40		8.02
7. Navy beans	9.40	3.00	5.80	8.20	6.43	3.88	3.42	3.21	0.49	6.00	84.80	4.21
8. Brewers' yeast (blend of three)	13.10	3.00	6.00	7.80	7.40	2.34	3.59	5.07	1.63	6.40	95.70	7.32
9. Torula yeast	8.60	2.80	5.50	8.30	6.84	2.62	4.55	4.97	0.83	5.90	92.50	7.58
10. "Kitchen Food" yeast	7.00	2.84	5.70	6.80	6.34	6.42	4.09	4.88	3.22	5.90	95.80	7.26
11. Corn germ (defatted)	10.10	3.60	4.10	7.70	5.17	4.60	2.83	4.22	3.60	5.90	92.60	3.68
12. Wheat germ (defatted)	9.60	4.29	3.80	6.60	5.11	2.80	4.08	2.97	2.91	6.30	93.30	6.35
13. Cotton-seed meal (defatted)	13.20	3.64	3.90	6.00	3.42	3.49	4.16	3.05	3.86	5.10	88.30	9.60
14. Peanut meal (defatted)	13.80	3.00	4.60	6.70	3.01	3.43	2.82	2.61	1.95	5.30	88.60	10.03
15. Sunflower seed meal (defatted)	11.30	2.96	5.10	6.80	3.02	3.35	4.96	3.41	2.38	5.60	88.10	8.73

[1] % of N of Whole Substance × 6.25.

adsorb the basic amino acids (approximately 100 mg washed adsorbent to 10 ml diluted amino acid solution), but difficulties were encountered in securing quantitative elution (G.R.B.).

The methods for arginine and histidine are satisfactory, however, without this additional check; for they give satisfactory recoveries of the pure amino acids added to the hydrolysates.

Lysine

This one of the basic amino acids has given more trouble than either of the others for securing satisfactory recoveries of known amounts of the pure substance added to the hydrolysates. The assumption that all of the nitrogen remaining in the catholyte after two or three electrodialyses, and precipitation of arginine and histidine, is nitrogen of lysine proved after prolonged trial to be wholly erroneous. Recoveries of pure lysine added to the hydrolysate never were satisfactory. Nor did any of the methods designed for direct application to the hydrolysates (without electrophoretic separation) which were tried prove satisfactory from this standpoint. Consequently resort was had finally to the decarboxylase method of Zittle and Eldred ('44) as confirmed by Neuberger ('45).

This method as modified slightly by one of us (L.E.E.) proved entirely satisfactory for recovery tests. The modification consisted only in running the reaction to completion. The acid tip method as described by Neuberger was used and should give at least 98% recovery.

Recoveries were run at least once, in triplicate or quadruplicate, often twice, on each food analyzed. The method used consisted in the mixing of equal quantities of the hydrolysate and the lysine standard, both containing the same quantity of lysine, as recommended by Dunn et al. ('44). The corrected readings of CO_2 obtained from the mixture on the Warburg manometers should equal one-half the readings obtained from the same volume of each component separately.

Amino acid mixtures without lysine produced no more CO_2 with the decarboxylase than the blanks.

% of N of Whole Substance × 6.25.

TABLE 4

Essential amino acids in fifteen protein foods expressed as percentages of the whole food.

	ARGININE	HISTIDINE	ISO-LEUCINE	LEUCINE	LYSINE	METHI-ONINE	PHENYL-ALANINE	THREO-NINE	TRYPTO-PHANE	VALINE
1. Whole egg	1.19	0.45	0.86	1.19	0.96	0.48	0.86	0.61	0.20	0.89
2. Beef (lean, round)	1.51	0.67	0.86	1.16	1.23	0.72	0.53	0.72	0.22	0.78
3. Horse meat (tough, stringy)	1.68	0.81	0.99	0.91	1.47	1.21	0.69	0.85	0.12	1.03
4. Halibut (large steaks)	1.68	0.74	1.24	1.76	1.90	0.89	0.68		0.22	1.20
5. Haddock (fresh, boneless)	1.80	0.64	1.22	1.67	1.86	0.79	0.81	0.76	0.24	1.14
6. Soy bean flour (fat extracted)	2.75	0.90	1.42	1.90	1.48	1.80	1.28	1.31	0.54	1.35
7. Navy beans	2.10	0.67	1.28	1.83	1.43	0.86	0.76	0.72	0.11	1.34
8. Brewers' yeast (blend of three)	5.70	1.31	2.63	3.42	3.24	1.02	1.50	2.23	0.71	2.80
9. Torula yeast	3.75	1.22	2.41	3.63	3.00	1.15	2.00	2.18	0.36	2.58
10. "Kitchen Food" yeast	3.06	1.23	2.48	2.96	2.76	2.77	1.78	2.12	1.40	2.56
11. Corn germ (defatted)	2.15	0.77	0.87	1.64	1.10	0.98	0.65	0.89	0.77	1.25
12. Wheat germ (defatted)	3.54	1.58	1.41	2.44	1.89	1.03	1.51	1.10	1.03	2.34
13. Cotton seed meal (defatted)	6.97	1.92	2.06	3.18	1.81	1.84	2.20	1.61	2.20	2.70
14. Peanut meal (defatted)	7.62	1.66	2.55	3.77	1.67	1.90	1.57	1.45	1.10	2.94
15. Sunflower seed meal (defatted)	5.46	1.43	2.78	3.71	1.45	1.61	2.39	1.64	1.14	2.70

Once the sample is freed of excess HCl it must be kept at low temperature (below 30°C.).

This method of determining lysine was checked against the microbiological method described by Stokes et al. ('45) (q.v. below). Table 1 exhibits the results from the two for the protein of the fat-free products and the whole foods as purchased or contributed.

Leucine isoleucine and valine

At the beginning of this work the oxidation method of Fromageot and Heitz ('39) as modified by Block, Bolling and Kondritzer ('40) was employed for determination of these three acids, but results were very variable. Later, even after improvements in technique were made by one of us (R.T.) it was found quite impossible to get satisfactory recoveries of pure substances added to the hydrolysates. The reason may be seen from the following:

By this procedure the branch-chained acids, after conversion to their hydroxy derivatives, yield acetone and the amount from each is readily measured. Since leucine and valine yield acetone but in different quantities depending on the conditions of oxidation, two different oxidative reagents, namely, buffered $KMnO_4$ and buffered $K_2Cr_2O_7$, are used on each protein hydrolysate. The percentage yield obtained directly on the pure substances with the two oxidants are then combined with the amounts of acetone from the hydrolysates to set up two simultaneous equations expressing the results of each oxidation (see Block and Bolling, '45, p. 224). From these equations the content of leucine and valine is a given hydrolysate may be calculated. Isoleucine gives 2-butanone (ethyl-methyl ketone) and its content is estimated in the same manner by the yields from known quantities of isoleucine. Careful examination of the procedure for leucine and valine, however, revealed that while with oxidation by $KMnO_4$ the yields of ketones distilling over from pure leucine and valine did not change when each was added to amino acid mixtures or to hydrolysates, with oxidation by $K_2Cr_2O_7$ the yields found

after addition of the pure acids to mixtures or to protein hydrolysates definitely decreased from the values obtained on the pure branch-chained acids. Such results would be expected to cause the calculated values for leucine to be too high and those for valine to be too low (i.e., with yields as found in this laboratory which differed always in the direction illustrated by the example; after $KMnO_4$ leucine 24%, valine 65%; after $K_2Cr_2O_7$, leucine 10%, valine 50%).

To avoid such discrepancies another method for these acids was sought and the microbiological method of Stokes and associates ('45) came to our attention.[6] Satisfactory control curves, close agreement between duplicate samples and good recoveries of the pure amino acids were experienced using the organism Streptococcus faecalis, as advocated by the authors.

A comparison of the values found for the three acids by the two methods — the micro-oxidative and micro-biological — may be seen for a dozen proteins in table 2. It is clear that the oxidative procedure gives a consistently higher content of leucine and a consistently lower content of valine than does the microbiological, just as predicted for calculated and true values from long experience by one of us (R.T.) with the micro-oxidative procedure. The percentages for isoleucine determined oxidatively fall on either side of, or coincide with, the values determined microbiologically.

The percentages for the three acids found for the fifteen food proteins and the corresponding whole foods reported in tables 3 and 4, respectively, are those obtained by the microbiological method.

Methionine

The determinations were made by the micro-chemical colorimetric method of McCarthy and Sullivan ('41) without modification. Pure crystalline methionine[7] added to the hydrolysates gave average recoveries of 97%.

[6] Through the good offices of Dr. C. D. Kochakian, Dr. Stokes very kindly furnished a description of their procedure previous to its publication.

[7] Merck.

Phenylalanine

This amino acid was determined by Block's ('38) adaptation of the Kapeller-Adler ('32) method and checked later by Block and Bolling's ('45, p. 109) adaptation of the Kapeller-Adler-Kuhn procedure (Block and Bolling, '45, p. 108). Recoveries of the pure amino acid added to the hydrolysates averaged 100% (range 87 to 109%).

Threonine

This determination was made by the periodate method of Shinn and Nicolet ('41) without modification. Average recovery of pure threonine was 98% (range 94 to 103%).

Tryptophane

The alkaline hydrolysis especially devised for determination of this amino acid was carried out as follows. The dried acetone-ether insoluble fraction showing negligible loss of nitrogen in the reagents was used. With fairly pure proteins like egg and the meats 70 mg samples were sufficient, but with the vegetables containing residues of roughage and starch larger samples were required. The appropriate amount of the dry material was weighed into 20 mm strong Pyrex test tubes and 2 ml of 5 N NaOH added per 70 mg of dry substance. The test tubes were then sealed with an oxygen-gas flame and heated at 115–116°C. for 16 to 18 hours in an oil-bath. In the autoclave 6 hours at 15 lbs pressure are sufficient. Some of the materials thus sealed up caused the tubes to explode. They were mostly materials of the vegetable type, but the horse meat used was particularly explosive. In these cases larger, or heavier-walled tubes had to be used. In the autoclave of course explosion is prevented by the outer pressure.

Tryptophane in samples hydrolyzed in the manner described was found to be stable for 24 hours. Check experiments, in which both 100% and 50% of the amount contained in the hydrolysate (determined colorimetrically) were added to the

dried material before hydrolysis, showed average recoveries of 99% and 98%, respectively.

The alkaline hydrolysate was neutralized with 20 N H_2SO_4 to a pH of 7.0.

The colorimetric method of Lugg ('37, '38) with the modification suggested by Brand and Kassell ('39) was used. However, in running blanks it was found that dilution of the hydrolysate with water gave results that were too variable, so the blank used was an exact duplicate of the unknown. Also in the final step 0.5 ml of water was used instead of the sodium nitrite. Otherwise the combined Lugg-Brand and Kassell procedure was unchanged. Average of recovery in the colorimetric determination was 95%.

SUMMARY AND CONCLUSIONS

The methods used, and controls employed for their verification, in the analyses of fifteen high protein foods for the ten essential amino acids are described. Several of the methods available in the literature at the time this work was undertaken (September, 1943) after thorough trial, had to be discarded as inadequate. This was notably true of the electrophoretic method for separation of the three basic amino acids as well as the current colorimetric procedure for these acids singly and the oxidative method for determination of isoleucine, leucine and valine. Criticisms of these methods are given.

Of the final results presented in table 3, those which apply to corn germ and beefsteak represent closely the values used in paper III of this series for making up the mixtures of the essential amino acids in imitation of these two proteins. The principal discrepancies concerned leucine, which was nearly equally too high (4–5%) and valine equally too low (1.3%) in both foods. The biological values of the mixtures in these two instances, after correction for the unnatural isomers, were brought close to those of the proteins they imitated.

All methods which could be controlled by recoveries of the pure amino acids were so controlled and no procedures were

adopted which did not give average recoveries of 95% or better.

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BIOLOGICAL VALUE OF PROTEINS IN RELATION TO THE ESSENTIAL AMINO ACIDS WHICH THEY CONTAIN

V. COMPARISON OF THE AVERAGE EFFECT OF TEN SINGLE AMINO ACIDS WITH EXTRA EGG PROTEIN AS SUPPLEMENTS TO AN EGG DIET [1]

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INTRODUCTION

Ever since the discovery by Weiss and Rapport ('24) that the specific dynamic effect of alanine and glycine is completely neutralized by feeding them together with casein or gelatin, it has been a problem to know whether this is a general phenomenon or one which occurs only with certain amino acids and proteins of a peculiar composition. We have not been able to find any confirmation of their findings from other combinations.

The most plausible explanation of this suppression of a thermogenic effect, among several which the authors suggest, it would seem, is the combination of alanine and glycine into peptides which are more readily retained than those formed from the protein alone. For if alanine or glycine were simply mixed with other free amino acids and so absorbed, unless

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Rochester.

there were polypeptide formation on the way to the liver (a very short journey), there should be more total amino acids brought under the deaminating power of the liver in a given time after feeding and therefore (probably) greater thermogenesis. In other words there would be a summation of the specific thermogenesis of amino acid with that of the protein.

Now if it be supposed that non essential amino acids can form such peptides with the products of proteolysis, so that they escape deamination (which presumably must precede the heat effect) how much more reasonable is it to suppose that essential ones would escape this fate, under such circumstances. Particularly would this seem to be a conservative measure if the supplemented protein were poor in essential amino acids. On the other hand supplementation of high biological value proteins with essential amino acids presumably would not be so likely to enhance retention, for that would be "to carry owls to Athens."

The specific thermogenic effects are secondary to our present interest. Work is in progress in the laboratory on this property of the essentials and will be reported upon in due time. However, the disclosure as to which if any of this group added as supplements to a high biological value protein, at a level of intake insufficient to establish nitrogen equilibrium, can or cannot enhance gross retention, or, still better, that more refined order of retention which is called biological value, would point the way to rewarding studies of thermogenic effects. Little is known of this aspect of amino acid metabolism. Is it all governed by what the tissues need or does the resistance to oxidative deamination or to transamination, operating in a purely chemical manner, play a part in the fate of a supplement? Schoenheimer and associates ('38, '40) have shown with isotopes N^{15} and deuterium that one of the essential amino acids, lysine, does not participate in the exchange of amino groups except as donor and once deaminated cannot be reaminated. This would appear to presage a good thermogenic effect: but Doty and Eaton ('37) already had shown that lysine has no specific dynamic effect in the dog.

Perhaps we may find that certain amino acids out of the group of essentials, precisely because they cannot readily become reaminated and so be isolated from tissues as such, nevertheless can readily be combined into peptides and consequently have reserved for them the more conservative and, shall we say more "noble" role of promoting synthesis. This kind of inquiry might well lead to a rating of the amino acids in a biological-value scale of their own.

It was natural for this laboratory to choose egg protein for such a study both because of its established superiority and because of the experience with it gained here from previous studies. Whole egg protein fed at a level of 5% of the calories alternatively with other proteins enables a diet squad, with other fundamental requirements met, to maintain a good nutritive condition for considerable periods of time. At this level it provides a good base of reference for relative biological values and at the endogenous level, or lower, it furnishes a suitable liason for the interconvertibility of biological values to the absolute scale (Murlin, Edwards, Hawley and Clark, '46b). The present problem required a level of intake lower than that necessary for nitrogen equilibrium.

The diet squad for this group of experiments was the first one organized for the program described by the general title of the series. It consisted of six men and four women ranging in age from 20 to 36 years, in weight from 45 to 83 kg and in height from 156 to 176 cm. They were selected from the undergraduate, graduate and medical students of the University and included one teaching and research assistant. All were in sound health at the beginning of the series, and, with the exception of three who fell victims of a prevailing epidemic of influenza and consequently were incapacitated during the last two periods and two others who partially succumbed to the epidemic for a day or two, all remained well and fit. There were some distressing symptoms from ingestion of two of the amino acids in particular, dl leucine and dl methionine, but these disappeared promptly when ingestion ceased (see table 3 for footnotes concerning these reactions).

PLAN AND PROCEDURE

Besides attempting to establish differences in retention potency, not to dignify the term too much, among the ten essential amino acids there was another objective; namely, to compare the combined "potency" of the several acids with that of a quantity of egg protein, likewise superimposed on the basal egg diet and supplying to all members simultaneously approximately the same quantity of essential nitrogen as the average supplied to all the ten subjects taking one amino acid each as supplement. Should the squad be able to tolerate as single amino acids the same quantity of nitrogen, say 1 gm, as the egg supplement contained, and continue so to do for an equal length of time, we should have what might be called a vertical measure as well as a horizontal one, of the effect on B.V. This would be the more convincing if two experiments with the single amino acids should give average results which agreed well. Furthermore, and this was the reason for beginning the general inquiry as we did, it would not be necessary to wait for completion of the analytical work on the egg protein. The experiment would be a test as to whether the whole of the nitrogen of the racemic forms or only that of the natural isomers should be used in such a comparison, when the correct analysis became available.

After a preliminary adjustment period of 4 days, the distribution of calories was fixed at protein 4, fat 45 and carbohydrate 51%, the total calories at 48 cal./kg of net body weight, and collections of excretion were started. In period II the egg nitrogen was continued at the same level, making this the definitive pre-experimental control period. In period III the egg nitrogen was increased for each person by exactly 1 gm, allowance being made in the carbohydrate for the 25 extra cal. from protein.

Period IV, the post-experimental control had to be at the same time the pre-experimental control for the first period of amino acid supplements because of time limitations. For this reason and in order to insure good retention of the amino acids it was desirable to start them from a state of assured

minus nitrogen balance. The basic intake level therefore was reduced in period IV by 0.31 gm N average, below that ingested in period II. The consequence was that the difference between the ingestion of the two control periods averaged together and that of the experimental period was increased to 1.16 gm N daily instead of the 1.0 gm originally intended. This made the nitrogen of the natural isomers of essential amino acids in the egg supplement as shown by later analyses 0.7 gm average daily (table 1) instead of the postulated 0.5 gm.

For the amino acid supplements this same level of basic egg protein was maintained through periods V to IX. The calorie distribution and the total calories also remained the same for each person except for very minor adjustments. Because of difficulty in tolerating certain of the amino acids experienced by four different subjects, the average nitrogen from all ten of the essentials actually ingested daily was 0.768 gm for the first experiment and 0.77 gm for the second (table 3) instead of 0.8 gm which would have been the average for both, if all had gone well. The average actual total nitrogen from natural isomers was 0.46 gm in both experiments.

METHODS

In the diet kitchen weighed samples of all foods, approximating the average amount ingested by the squad members daily, were assembled in tightly sealed jars and kept in the refrigerator. The eggs always were as fresh as could possibly be purchased under war restrictions and were delivered from the farm directly to the hospital. They were freshly broken, homogenized and kept stirring while being sampled for each person's allowance at the different meals.

All items of the diet were regularly analyzed for nitrogen content, samples being taken from the homogenized material, either while the Waring, or other type of blendor, was in motion; or, for repeats, after thorough mixing. The macro Kjeldahl method was more suitable for these food products than the micro methods and therefore was used for the excreta. Feces were separated into periods by ingestion of a

capsule of carmine taken immediately before the first breakfast of each new period. In this particular series aliquot samples by weight were taken from each stool while fresh and the aliquots for each subject assembled in a jar of adequate size for use of the Waring blendor. In later series it was found that each subject's feces could be collected for the entire period in a 1-gallon "candy jar" placed directly in the commode. The jar contained a 5% solution by weight of nitrogen-free H_2SO_4 with a trace of copper sulfate dissolved in it to precipitate the H_2S. A special rotary motor provided with paddles constructed after the Waring design but more resistant to the acid was used to homogenize the stools at the end of each period. The contents always were freshly stirred for sampling.

Once started, continuous nitrogen balances were recorded for every subject.

RESULTS

From our best analysis (Edwards, Sealock, Bartlett et al., '46) for the ten essential amino acids in whole egg, repeated in table 1, may be calculated the average amount of each contained in the control periods II and IV as compared with the total of each in period III when the extra egg was ingested.

It appears that in the experimental period each person ingested 58 gm extra whole egg substance containing 4.47 gm of the ten essentials. All the other constituents of the diet remained unchanged. The essentials made up 61.6% and the non essentials 38.4% of the 7.25 gm total protein represented by the 1.16 gm nitrogen.

In table 2 are seen the condensed data concerned in the effect of this egg supplement on the individual nitrogen balances for the three periods. The gross additional nitrogen retentions, calculated for each person on the basis of his own extra protein ingestion, were found to be very variable — from 38 to 98%. It is of interest to note that the average ingestion of 1.10 gm extra nitrogen (an increase of 28%) by four women produced an average improvement in nitrogen balance (gross retention) of 0.945 gm or 86% of the extra ingestion;

TABLE 1

Average amounts of the ten essential amino acids contained in the whole egg substance fed in periods II and IV compared with period III.

	IN WHOLE EGG	AV. IN PERIODS II AND IV	IN PERIOD III	DIFFERENCE $\text{III} - \frac{(\text{II} + \text{IV})}{2}$	
	gm/100 gm	*gm/206 gm*	*gm/264 gm*		
Arginine	1.19	2.45	3.14	0.69	
Histidine	0.45	0.92	1.18	0.26	
Isoleucine	0.86	1.77	2.27	0.50	
Leucine	1.19	2.45	3.14	0.69	
Lysine	0.96	1.98	2.54	0.56	
Methionine	0.48	0.99	1.27	0.28	
Phenylalanine	0.86	1.77	2.27	0.50	
Threonine	0.61	1.26	1.61	0.35	
Tryptophane	0.20	0.41	0.53	0.12	
Valine	0.89	1.83	2.25	0.52	
Total	7.69	15.83	20.30	4.47	= 0.7 gm N

Average extra natural isomers ingested in period III, 4.47 gm.
Average extra whole egg nitrogen ingested in per. III, 1.16 gm.

TABLE 2

Effect of 1.16 gm extra egg N on the nitrogen balances, average daily (gm).

SUBJ. AND SEX	CONTROL II (6)[2]	EXP. III (5)	CONTROL IV (6)	AV. OF II AND IV	GAIN OF III	% OF EXTRA N RET.[1]
1 ♂	— 0.035	+ 0.497	— 0.166	— 0.100	+ 0.597	54
2 ♀	+ 0.501	+ 1.181	— 0.354	+ 0.073	+ 1.108	98
3 ♂	+ 0.551	+ 0.627	— 0.200	+ 0.175	+ 0.452	38
4 ♀	+ 0.469	+ 1.158	— 0.224	+ 0.122	+ 1.036	92
6 ♀	+ 0.599	+ 0.958	— 0.297	+ 0.151	+ 0.807	80
7 ♂	+ 0.066	+ 0.226	— 0.701	— 0.317	+ 0.543	48
8 ♂	— 0.084	+ 0.145	— 1.192	— 0.638	+ 0.783	67
9 ♂	— 0.823	+ 0.336	— 0.884	— 0.853	+ 1.189	89
10 ♀	— 0.434	+ 0.312	— 0.602	— 0.518	+ 0.830	74
11 ♂	+ 0.158	+ 0.801	— 0.248	— 0.045	+ 0.846	71
		+ 0.624		— 0.195	+ 0.819	
				Average		71%
				Average 4 F's		86%
				Average 6 M's		61%

[1] Calculated on individual differences between N fed in periods II and IV (average) and that fed in period III. This difference (av. for all 10 subjects) was 1.159 gm. It varied from 1.004 gm for subj. 6 to 1.334 gm for subj. 9.

[2] Figures in () indicate no. of days in the periods.

while the average extra ingestion of 1.2 gm (26%) by six men produced an improvement of only 0.730 gm or 61% of extra nitrogen ingested. Only 2% difference in the extra ingestion made a difference of 25% in gross retention. It appears that women are better conservators of nitrogen. The average for the whole group without regard to sex is 0.819 gm or 71% of 1.16 gm extra nitrogen.

Taking the basic egg protein as having a biological value of 100% and rating the augmented amount in period III against this base, it is found that the biological value is 93.1% — a reduction of 7%. But the difference between the sexes is not so great as for percentages gross retention: viz. females 93.3, males 90.6 (table 4). Of course from the nature of the calculations it could not be so great.

The average extra amount of whole egg substance (58 gm) is not far from the amount supplied in one large White Leghorn egg. The basal level is represented approximately by four medium sized eggs. Hence if the average person were subsisting on four eggs a day as his sole source of protein in a diet containing adequate calories, the addition of one large hen's egg would assuredly put him well over the equilibrium line: for he would retain some 70% of the extra egg protein (0.8 gm N) and this would provide him with a composite of the ten essentials weighing 4.47 gm, and making his total supply for the day 20.3 gm (table 1). We must not overlook the fact that 38.4% of the egg protein consists of the non essential amino acids, and this experiment obviously throws no light on which of them is retained. Supplementary egg protein, however, is not so efficiently utilized for maintenance of live tissue or for deposit as reserve protein, as the case may be, as is the submaintenance supply represented by the basal amount; hence the lower B.V.

Single essential amino acids as supplements

These experiments were carried out in the same manner as the single one above, i.e., the periods were arranged in the same order (IV, V and VI making one cycle and VI, VII and

IX constituting a second, each with the experimental embraced between two controls). But instead of administering the same supplement to all members of the squad simultaneously they were each given a single and separate amino acid (one of the ten to each of the ten), in an amount calculated to supply 0.5 gm N from the natural isomer alone. All the dl forms except tryptophane supplied 1.0 gm N and all the others 0.5 gm, tryptophane included because, the evidence available at this time indicated that both l and d isomers of this essential were nearly equally well utilized (retained) by man.

In table 3 are presented the essential data for calculation of the percentages of the actual amino acid nitrogen ingested which were retained in the two experiments. Considering the vicissitudes which the squad members suffered from the distasteful qualities of some of the amino acids and from influenza (see footnotes) it is surprising how nearly equal the average figures are for amounts ingested and retained [2] in the two experiments. The credit is due to the pluck and persistence of the squad members and the competence of the assistants in the dietetic and analytical work. The hope for an agreement between these two experiments was realized adequately to form some basis of opinion as to the comparative benefits one may expect from natural and artificial supplements supplying essential amino acids.

The average retention, both in weight and as percentage of the amount of nitrogen ingested, is as nearly equal in the two experiments as could be expected. The average improvement in nitrogen balance compared with the average improvement from the egg supplement (71% of ingested) is not disappointing when we remember that egg supplies nothing but natural isomers whereas the average nitrogen from natural

[2] It should be stated that the calculation for percentage of amino acid nitrogen retained in each instance was based on the full 6 days of each period concerned, except for subjects P.F. and A.L. in period V of the first experiment and subjects J.A. and P.M. in period VII of the second experiment for whom the calculations included only the first 2 full days and any additional days for which correction could be made. Reasons for these omissions from the periods named are fully explained in the footnotes to the table.

isomers supplied by the synthetic amino acids is only 4.46 gm or 60% of the total. When the retention, or improvement in nitrogen balance, is calculated on this basis the percentage is 91 and 93% for the two experiments, respectively. Clearly it is the natural isomer in the dl forms one must count on to match the effects on nitrogen balance from comparable amounts of nitrogen in natural foodstuffs (see paper III, Murlin, Edwards, Fried and Szymanski, '46a).

It is evident from table 3 that the three basic amino acids produced high retentions in both experiments; that isoleucine and leucine produced one low and one high in each — the high by the woman of the pair in both and low by the man; that phenylalanine and valine produced moderate retentions, each yielding a value above 50% and each yielding one in the neighborhood of 20 to 30%, the man of each pair scoring the higher figure; and finally, that methionine, tryptophane and threonine yielded retentions so low as to suggest that the egg protein already contained all of these three that the body could utilize to advantage.

For the performance of the two sexes we should include only the eight amino acids that were used as supplements by both. Thus it is found that the eight tests on women produce the average retention of 84%, the eight on men 42% — just half as much. The difference is greater than with egg protein as the supplement. This striking sex difference can scarcely be explained by greater tolerance by the women; for the evidence, though not a little complicated by influenza symptoms, is the other way. It seems more probable that the men may either metabolize the amino acids more rapidly by having a greater potential for oxidative deamination or that they may excrete them unchanged more rapidly (see below under biological values).

Biological values

There are three points of interest in this effect of the amino acid supplements: (1) the comparison of the two experiments; (2) comparison of the sexes and (3) the effect of the amino

TABLE 3

Summary of N ingestion and N retention from each essential amino acid added as supplement to egg diet.

BOTH EXPERIMENTS	FIRST EXPERIMENT, PERIODS IV, V, VI				SECOND EXPERIMENT, PERIODS VI, VII, IX			
AMINO ACIDS	Subject and sex	gm N ingested	gm N retained	% retained	Subject and sex	gm N ingested	gm N retained	% retained
l (+) arginine	T.L. ♂	0.50	0.377	75	M.G. ♀	0.50	0.628	125
l (+) histidine	M.M. ♂	0.50	0.518	103	J.S. ♂[3]	0.50	0.539	108
l (+) lysine	M.G. ♀	0.50	0.921	184	T.L. ♂	0.50	0.738	147
dl isoleucine	E.L. ♂	1.00	0.330	33	P.F. ♀[3]	1.00	1.057	106
dl leucine	P.F. ♀	0.89[1]	1.597	180	E.L. ♂	1.00	0.196	20
dl methionine	A.L. ♂	0.79[2]	—0.242	—30	J.A. ♂	0.77[4]	0.138	18
dl tryptophane	J.S. ♂	0.50	—0.060	—12	P.M. ♀	0.43[5]	0.101	23
dl phenylalanine	P.M. ♀	1.00	0.208	21	A.L. ♂	1.00	0.524	52
dl threonine	V.V. ♀	1.00	—0.002	0	M.M. ♂	1.00	0.030[6]	3
dl valine	J.A. ♂	1.00	0.539	54	V.V. ♀[3]	1.00	0.313	31
Average		0.768	0.419	54.5		0.77	0.426	55.4

[1] This subject had nausea headache, heart palpitation, abdominal cramps. Took amino acid only 3 days — correction for amt. not taken third day.

[2] Subject had nausea, weakness, dizziness. Correction for amt. not taken third day (the last).

[3] These three subjects absent from second control period IX.

[4] Subject had nausea, dizziness, weakness. Continued to 4½ days, but omitted part on fourth day. Correction for this.

[5] Symptoms of influenza; also nausea and vomiting. Correction for amt. lost 1 day.

[6] Subject confined to bed with influenza last 2 days of period VI. Average used for first 4 days.

acids on the fecal nitrogen excretion. To discuss these intelligently requires a display of all the data for evaluation of biological values, as given in table 4.

The average figures at the end of the table show that the effect of these several supplements taken together on "biological value"[3] of the egg protein is an experiment which can be readily duplicated. However, there is one criticism that can fairly be raised; namely, the absence of three members of the squad from the second control period IX. Study of the original nitrogen balance data fails to reveal any feature of this period which disqualifies it for standing as a control for the remaining seven members, notwithstanding that the intervening period VIII[4] postponed the control 6 days; for they were in precisely the same average state of balance for undertaking the second control as if they had started it 6 days earlier. It is practically certain that the same would have been true of all ten if it had been possible for them to continue to the end. The only consideration remaining then, is whether period VI alone as control would give the same average re-

[3] It will be understood that this is not a true or absolute biological value in the conventional sense but an effect on the percentage of the absorbed which is retained, compared with egg unsupplemented, in other words, a relative B.V.

[4] Period VIII represented an attempt to profit by the high retentions shown in the first experiment (and indicated by early analysis of the urine in the second) by feeding all members the five amino acids which had exhibited retentions higher than 50% and to furnish all the supplement from these alone in combination. The mixture contained: 3.76 gm l (+) arginine; 28.69 gm l (+) lysine, 14.97 gm l (+) histidine — all as the monohydrochlorides — also 18.74 gm dl leucine and 16.74 gm dl valine, calculated to supply 1.2 gm total nitrogen to each of eight subjects remaining at that time, and so proportioned as to provide 1.0 gm nitrogen from natural isomers, from the five, in the order named as follows: 0.1, 0.4, 0.3, 0.1, and 0.1 gm. This mixed supplement to the same egg diet as used in periods VI and IX was continued for 3 days, when the leucine was dropped (because of some symptoms ascribed to it) and the remaining four amino acids were readjusted to supply 1.1 total nitrogen per person, containing 1.0 gm N from natural isomers in the following proportions: arginine 0.2, lysine 0.4, histidine 0.3 and valine 0.1 gm.

The average retentions from the seven members who completed this period and period IX calculated against the nitrogen balances of the same periods VI and IX as controls was 43% from the mixture of five and 35.3% from the mixture of four amino acids. Evaluated on the basis of nitrogen of natural isomers only these values became 52 and 42%, respectively.

sults for percentage retention and biological value for the remaining seven as do the controls VI and IX. If so it is clear that the inclusion of the results for the three members having only one control is valid. The answer is that the average nitrogen retention and the average B.V. would have been some 4% higher and 2.8% (points of B.V.) lower, respectively, than those averages for the seven (not shown in table) now are. In other words the present average retention for the second experiment (table 3) is probably 1.3% too high and that for average B.V. (table 4) is about 1.1% too low (remembering that B.V. is always a percentage). These obviously are not significant errors incurred by using results for the three subjects who had only one control period.

The difference between the average biological values for men and women, 90.6 ± 7.06 and 93.3 ± 10.14, respectively, is too small to be statistically significant as is evident from the standard deviations and the small number of observations. It would not require more than twice the number, with no greater variation, to produce a difference between means which would be significant. The reasons for such an outcome judging by results already obtained, would be: (1) That women have a greater average correction on the fecal nitrogens, as will be evident in casting the eye down column C of table 4; while (2) men have a greater average correction on the urinary nitrogens, as seen in column I. The former being negative increases the nitrogen absorbed and thereby tends to decrease B.V., but the difference is slight; while the larger correction on the urine, being positive has a greater effect to reduce the amount of nitrogen retained by men and therefore to reduce B.V. still more. These effects are evident from the averages (at the bottom of the table) for the two groups of eight tests on men and eight on women taking the same amino acids.

Effect of amino acids on fecal nitrogen

In fifteen out of twenty comparisons in table 4 of the fecal nitrogens from the unsupplemented egg diet and the supple-

TABLE 4

Biological value of egg protein and individual amino acid supplements in terms of egg alone.

AMINO ACID SUPPLEMENT AND SUBJECT	CONTR. PERIOD	EXPL. PERIOD	CONTR. PERIOD	A EGG AND A.A. FECAL N	B EGG ALONE FECAL N	C A–B	D EGG AND A.A. FOOD N	E ABSORBED N D–C	F TRUE DIGEST. %	G EGG AND A.A. URINE N	H EGG ALONE URINE N	I G–H	J $\frac{E}{I \times 100}$	K B. V. (100–J)	SEX
Valine J.A.	V	IV	VI	1.112	1.301	—0.189	5.217	5.406	104	3.804	3.181	0.623	11.5	88.5	♂
Valine V.V.	VII	VI	[1]	0.674	0.550	+0.124	4.695	4.571	97	3.588	3.025	0.563	12.3	87.7	♀
Leucine P.V.	V	IV	VI	0.740	0.866	—0.126	5.232	5.358	102	3.470	4.050	—0.580	—10.8	110.8	♀
Leucine E.L.	VII	VI	IX	1.578	1.187	+0.391	5.493	5.102	93	4.051	3.638	0.413	8.1	91.9	♂
Methionine A.L.	V	IV	VI	0.699	1.068	—0.369	5.538	5.907	107	5.083	3.675	1.408	23.8	76.2	♂
Methionine J.A.	VII	VI	IX	1.273	1.408	—0.135	4.991	5.126	103	3.944	3.163	0.781	15.2	84.8	♂
Lysine M.G.	V	IV	VI	0.604	0.904	—0.300	4.851	5.151	106	3.459	3.580	—0.121	—2.3	102.3	♀
Lysine T.L.	VII	VI	IX	0.855	0.938	—0.083	4.318	4.401	102	3.256	3.441	—0.185	—4.2	104.2	♂
Arginine T.L.	V	IV	VI	0.954	0.979	—0.025	4.319	4.344	101	3.816	3.667	0.149	3.4	96.6	♂
Arginine [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Histidine J.S.	VII	VI	[1]	0.739	0.735	+0.004	5.006	5.002	100	4.505	4.548	—0.043	—0.8	100.8	♂

Arginine T.L.	V	IV	VI	0.954	0.979	—0.025	4.319	4.344	101	3.816	3.667	0.149	3.4	96.6	♂
Arginine [illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Histidine J.S.	VII	VI	[1]	0.739	0.735	+0.004	5.006	5.002	100	4.505	4.548	—0.043	—0.8	100.8	♂
Isoleucine E.L.	V	IV	VI	1.231	1.280	—0.049	5.493	5.542	101	4.484	3.764	0.720	13.0	87.0	♂
Isoleucine P.F.	VII	VI	[1]	0.746	0.740	+0.006	5.342	5.336	100	4.315	4.396	—0.081	—1.5	101.5	♀
Phenylalanine P.M.	V	IV	VI	0.609	0.669	—0.060	4.220	4.280	101	3.737	2.885	0.852	20.0	80.0	♀
Phenylalanine A.L.	VII	VI	IX	0.749	1.050	—0.301	5.745	6.045	105	4.461	3.684	0.777	12.8	87.2	♂
Tryptophane J.S.	V	IV	VI	1.196	0.922	+0.274	5.006	4.732	94	4.810	4.524	0.286	6.0	94.0	♂
Tryptophane P.M.	VII	VI	IX	0.634	0.683	—0.049	3.647	3.696	101	3.257	2.877	0.380	10.3	89.7	♀
Threonine V.V.	V	IV	VI	0.550	0.593	—0.043	4.693	4.738	101	4.199	3.154	1.049	22.1	77.9	♀
Threonine M.M.	VII	VI	IX	0.698	0.815	—0.117	5.875	5.992	102	5.452	3.974	1.478	24.7	75.3	♂
Averages				0.847	0.919	—0.072	4.976	5.048	101.4	4.072	3.638	0.434	8.4	91.6	
Average of minus values						—0.149		Average for Exp. 1						91.2	
								Average for Exp. 2						91.9	
Averages for the tests on 8 men and 8 women taking the same amino acids.			♀	0.640	0.738	—0.098	4.691	4.789	102	3.718	3.436	0.282	6.7	93.3	
			♂	1.046	1.059	—0.014	5.183	5.197	100	4.267	3.722	0.534	9.4	90.6	

[1] Only one control period on account of influenza.

mented one the nitrogen from the latter is less than that from the former, notwithstanding that for the entire 3 to 6 days of ingestion this diet contained anywhere from 0.43 to 1.0 gm more nitrogen daily. Does this mean that the presence of these amino acids directly stimulates additional absorption of egg protein over the amounts absorbed in the fore and after periods? Or does it mean that when the free amino acid is taken in considerable quantity (from 1.88 gm arginine to 11.8 gm phenylalanine) every day it acts either before absorption or after it enters the blood to increase secretion from the alimentary glands thereby reducing the amount of food nitrogen excreted? Preliminary experiments [5] in this laboratory indicated strongly that single amino acids dissolved in salt solution and infused slowly into the vein of an anaesthetised dog enhanced quite definitely after a short latent period the action of secretion, injected a short time before, to increase pancreatic secretion. There was also in a few experiments a somewhat reduced content of trypsin in the secretion collected during the augmented flow. This latter observation is in accord with those of Grossman, Greengard and Ivy ('43) on the trypsin content of rat pancreases resulting from the substitution of hydrolyzed casein [6] for casein in the rat's diet for 21 days. Increased flow of pancreatic juice, if continued for only 6 days might however go on without change of concentration of the enzyme, or produce proportionally less decrease in concentration, and so, because of better mixture with the alimentary contents, result in more complete digestion of the egg protein, sufficient to reduce the nitrogen content of the feces to the extent noted.

SUMMARY AND CONCLUSIONS

A comparison was made of the average effect on nitrogen balance of feeding to each of ten members of a diet squad a single but different one of the ten essential amino acids, with the effect of a small amount of whole egg protein added as a

[5] Performed by D. W. Kramer, C. J. Scarpellino and E. K. Ryder under direction of J. R. M.

[6] Amigen.

supplement to the same diet containing already an inadequate amount of this protein. The average total nitrogen from the amino acids fed singly as supplements should equal the nitrogen of the egg supplement. Actually owing to some vicissitudes experienced in taking some of the amino acids and the exigencies of time this ideal was not realized very exactly. Two experiments on the amino acids with different persons taking each one gave good agreement and furnished what may be called a vertical average in comparison with a horizontal (simultaneous) one on the egg supplement.

Another objective was to learn something of the relative retention potency of the several essentials and whether the total nitrogen of the racemic forms should be reckoned against the egg nitrogen or only that of the natural isomers.

The results were: (1) That the supplement of egg (learned from analyses obtained later) supplied 0.7 gm essential amino acid nitrogen of natural form, while the ten synthetic essentials supplied on the average only 0.46 gm from natural isomers. (2) That the average retention of the egg nitrogen was 0.819 gm or 71% of the total nitrogen fed, indicating that some non essential nitrogen must have been retained; while the average retention from the ten essentials was, in two experiments, 0.419 and 0.426 gm or 54.5 and 55.4%, respectively, of the average total nitrogen fed in the essentials, indicating a large wastage of nitrogen from the amino acids. (3) Calculated on the basis of the nitrogen from the natural isomers only the retentions in the two experiments were 91 and 93%, respectively. (4) The effects on biological value relative to the egg basal diet were about the same from the two types of supplement; namely a reduction of seven points in the percentage scale.

Other observations were that women appear to be better conservators of nitrogen than men, and that in fifteen of twenty tests the presence of amino acids in the diet reduced the fecal nitrogen, notwithstanding that each subject ingested from 0.43 to 1.0 gm more nitrogen than from the basal egg diet alone.

Conclusions reached were: (1) That a vertical average can be duplicated satisfactorily and furnishes much useful information, but would be still more useful after a correct analysis of the protein was in hand. (2) That the individual essential amino acids differ greatly in retention potency under the conditions of these experiments; and (3) that it is the nitrogen of the natural isomers which must be counted on to equal the effects on nitrogen balance of natural proteins.

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REPRODUCTION AND LACTATION STUDIES WITH RATS FED NATURAL RATIONS[1]

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In studies of the dietary insufficiency of a corn-soybean ration for swine, Ross ('43), Ross et al. ('44), Cunha ('44), and Cunha et al. ('44) found that a ration composed of 76.35% yellow corn, 17.50% soybean oil meal, 5.0% alfalfa meal, and 1.15% minerals failed to support normal reproduction and lactation. Normal reproduction and improved lactation resulted, however, when the basal ration was supplemented with 10% additional alfalfa meal. Certain vitamins, commercial preparations, and other feeds were shown to be ineffective supplements. These workers concluded that good quality alfalfa meal carried a factor or factors which wholly, or in part, corrected the deficiencies of the basal ration. It was also demonstrated that the ration which sows received during growth markedly influenced subsequent performance in reproduction and lactation.

Ross ('43) and Cunha ('44) report that similar corn-soybean oil meal-alfalfa meal rations did not support optimum growth in the rat. The addition of all the ten known B-complex vitamins did not stimulate growth to any appreciable

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extent. Cunha ('44) states that the possibility of a vitamin imbalance, or of some effect on intestinal synthesis or absorption, should not be overlooked, but suggests that some unidentified factor or factors were still needed to adequately supplement the basal ration for growth. Alfalfa, acid-washed casein plus choline, tankage, brewer's yeast, or 1:20 liver powder supplemented the basal ration. Alcohol-washed casein plus choline, vitamins fed singly or in combination, and certain other natural materials were ineffective supplements. Very little work has been done on studying the reproduction of the rat on a natural ration made up entirely of concentrates of plant origin.

We have studied reproduction and lactation in rats fed similar corn-soybean oil meal-alfalfa meal rations. An assay for determining supplements to the basal ration which would promote normal reproduction and lactation has been developed. Certain feeds and purified materials have been tested. The results of these experiments are reported in this paper.

EXPERIMENTAL

Females of Sprague-Dawley breeding were used. In some experiments the females were started on their rations at weaning, but in other experiments they were fed as indicated. Females were mated at 12 or 14 weeks of age. Males from the stock colony were used and were replaced each week by new stock colony males. All groups of females were kept in wire cages and fed ad libitum. The pregnant females were removed from the larger colony cages to smaller individual wire cages without false bottoms. Wood shavings were used for nesting. Two days after parturition all litters were reduced to six young. The length of the lactation period was 21 days and all litters reaching this age were weaned.

The basal ration was composed of ground yellow corn 75.34%, soybean oil meal (expeller process) 17.50%, alfalfa meal 5.0%, $CaHPO_4$ 1.0%, $CaCO_3$ 0.65%, NaCl (iodized) 0.50% and $MnSO_4$ 0.01%. Two drops halibut liver oil were given weekly by dropper. All major supplements to the basal

ration were made at the expense of the corn. Rations were mixed every 2 weeks and were stored at room temperature except in the case of high fat, or high mineral rations. These latter rations were stored in the dark at refrigerator temperatures.

RESULTS

Experiment I

The first experiment was designed to study the effect of the basal ration on reproduction and lactation in the rat. All females were fed the basal ration during growth and reproduction. Results are summarized in table 1.

TABLE 1

The effect of the basal ration on reproduction and lactation.

Total number females	19
Number sterile	7
Resorptions and toxemic deaths	4
Number litters born	8
Number live young	33
Number dead young[1]	6
Number young given to females to raise	27
Number young weaned	0

[1] Only those young found with the female are tabulated. Many were known to have been eaten by the female.

A large percentage of the females fed the basal ration were sterile. A peculiar type of hemorrhage was produced in the fetal attachments of the pregnant rats fed such a ration and resulted in expulsion of the dead fetus at term, or death of the fetus, attempted resorption, or toxemia and death of the female. Four of the females fed the basal ration died during or shortly after parturition. The breakdown of the capillaries of the cotyledons in many cases occurred during the early part of the gestation period as indicated by a bloody discharge by the pregnant female. In this case when the capillary breakdown occurred early, the rat was able to resorb the developing embryos. However, if this condition occurred a day or two before parturition, the female was not able to resorb the

dead embryos and as a result gave birth to dead young, or died from toxemia. Although these symptoms are similar to those described for a vitamin E deficiency, α-tocopherol supplementation (4 mg daily) was without effect. Young that were born alive appeared normal at birth, but did not live more than 1 or 2 days following parturition. Although the young attempted to nurse, no milk could be found in their stomachs. When this condition was observed in these experiments it was considered to be due to lactation failure.

Experiment II

Experiment II was designed to study the effect of previous dietary treatment upon reproduction and lactation in the rat. All females received the basal + 10% alfalfa meal ration during attempted reproduction and lactation. Rations fed prior to this period are indicated in table 2 where results are summarized.

TABLE 2

Effect of previous ration on reproduction and lactation.

LOT	RATION FED PREVIOUS TO ATTEMPTED REPRODUCTION	RATION FED DURING ATTEMPTED REPRODUCTION	TOTAL NO. FEMALES	NO. LITTERS BORN	NO. LIVE YOUNG	PERCENTAGE OF YOUNG WEANED
1	Basal ration (birth to breeding)	Basal + 10% alfalfa	11	8	50	0
2	Basal ration (weaning to breeding)	Basal + 10% alfalfa	8	7	39	25
3	Basal + 10% alfalfa (second generation)	Basal + 10% alfalfa	9	8	22	71
4	Basal + 10% alfalfa (weaning to breeding)	Basal + 10% alfalfa	17	17	86	86

Complete failure in lactation resulted in lot 1 when the females were raised from birth to breeding on the basal ration. The females of lot 2 were fed the basal ration from weaning to reproduction. This group weaned 25% of the young which were born alive. The females of lot 3 were fed only the basal + 10% alfalfa meal ration and were raised from females

that received a similar ration during reproduction and lactation. The females of lot 4 received only the basal + 10% alfalfa meal ration but were raised from females that received a stock ration [2] during reproduction and lactation. The second generation performance of females on the basal + 10% alfalfa meal ration (lot 3) was not as good as the first generation performance (lot 4). In the latter group, 86% of the young were weaned. The basal plus 10% alfalfa meal ration was in this case a borderline carrier of the factors needed to supplement the basal ration. These data indicate that the factors needed for reproduction and lactation can be stored by the female rat.

Experiment III

The effect of adding practical supplements to the basal ration was studied next. Females were raised until 10 weeks of age on a stock ration.[2] At this time they were placed on the basal ration for a 2–4 week "depletion period." At the end of the "depletion period" the females were placed on experimental rations. After a 2-week period, they were mated. Using this procedure it was possible to study the effects of adding some natural supplements to the basal ration. Results are summarized in table 3.

Reproduction on the basal ration was again very poor but somewhat better than had been obtained in experiment I. The addition of 10% alfalfa meal to the basal ration resulted in marked improvement in reproduction. Forty litters were born from forty-nine females, and the number of resorptions and sterile animals was reduced when 10% alfalfa meal was added. Brewer's yeast, tankage, soybean lecithin, a combination of casein (crude or acid-washed) plus choline, and 1:20 liver powder when added to the basal ration, also caused a reduction in the number of resorptions and the number of sterile females.

[2] Stock ration composed of ground yellow corn 70.0%, linseed oil meal 14.0%, casein 5.0%, alfalfa 4.0%, liver 2.0%, butter 3.0%, $CaCO_3$ 1.0% and NaCl (iodized) 1.0%.

Lactation performance was very poor on the basal ration; only 3% of the young given to the females to raise were weaned. The addition of 10% alfalfa meal or of 5.0% 1:20 liver powder markedly increased the percentage of young weaned. Results using a limited number of animals suggest that fish meal, soybean lecithin or a combination of casein (crude or acid-washed) plus choline furnished a factor or factors which also improved lactation performance.

TABLE 3

Effect of natural materials on reproduction and lactation.

BASAL RATION SUPPLEMENT	TOTAL NUMBER FEMALES	RESORPTIONS	NUMBER LITTERS BORN	NUMBER YOUNG GIVEN TO FEMALES TO RAISE	NUMBER YOUNG WEANED	PER CENT YOUNG GIVEN TO FEMALES THAT WERE WEANED
Basal ration only	65	19	35[1]	115	3	3
Alfalfa meal 10.0%	49	1	40	158	80	51
Brewer's yeast 5.0%	11	0	10	38	12	32
Tankage 5.0–10.0%	25	1	20	99	24	24
Fish meal 5.0%	7	1	4	21	21	100
Fish "press H_2O" 5.0%	4	1	2	11	3	27
Wheat 75.35%	9	1	8	46	3	7
Soybean lecithin 0.3%	4	0	4[1]	7	5	71
Casein (crude or acid-washed 5.0%) + choline (0.3%)	11	2	8	36	22	61
Casein (alcohol extracted, 5.0%) + choline (0.3%)	8	4	2	12	3	25
1:20 liver powder 5.0%	22	2	16	74	51	69

[1] Three litters born dead.

Wheat and a combination of alcohol-extracted casein + choline were ineffective supplements. Other materials not included in table 3 that were tested and shown to be ineffective supplements include wheat bran, wheat middlings, dehydrated oat grass, wheat germ, wheat germ oil and wheat germ meal. Six to ten females were used to test each of these materials not included in table 3.

DISCUSSION

Very little work has been reported on reproduction and lactation of rats fed all plant rations.

Various experiments with rats have shown that cystine and methionine effectively supplement certain soybean rations (Hayward et al., '36; Evans and McGinnis, '46). Other studies have shown that sulfur-containing amino acids promote lactation in rats when fed rations in which the protein is derived from alfalfa leaf meal (Wright and Haag, '39).

Alfalfa meal has been shown by Ross et al. ('44) and Cunha et al. ('44) to aid reproduction and lactation in swine fed rations similar to the basal ration used in the studies reported in this paper. Fairbanks et al. ('45) have reported that the addition of 10% alfalfa meal to certain natural rations containing adequate amounts of nutrients known to be required by the pig, resulted in effective gains and decreased death losses in young pigs.

The addition of certain natural and purified materials to the basal corn-soybean oil meal-alfalfa meal rations markedly improved reproduction and lactation. Alfalfa meal and 1:20 liver powder were effective supplements. Limited numbers of animals suggested that certain other materials, including combinations of casein (acid-washed or crude) plus choline, favorably improved the reproduction and lactation performance. Alcohol-washed casein, a relatively pure protein, plus choline was found to be an ineffective supplement. Further experiments are now being conducted to determine whether the factor or factors necessary to supplement the basal ration are of a known or an unknown nature.

The possibility of a vitamin imbalance or of some effect on intestinal synthesis or absorption should not be overlooked. Experiments have indicated that certain dietary factors not required in the diet when purified diets are fed, may be required when rats are fed certain soybean rations (Cunha et al., '43; Spitzer and Phillips, '46). Patton et al. ('46) suggest that sardine fish meal contains a factor which will stimulate growth in chicks fed a corn-soybean diet and that the need for this factor may be a peculiarity of the corn-soybean diet.

Results of experiment II show a marked difference in reproduction and lactation depending upon past dietary history of the females. This is also indicated when experiments I and III are compared. Although the basal females in both of the latter experiments failed to lactate, reproduction was somewhat better in experiment III where the rats had received a stock diet during growth. Thus it was demonstrated that a storage of dietary essentials needed for reproduction and lactation takes place with the rat. These results are in agreement with swine experiments of Ross ('43), Ross et al. ('44), Cunha ('44), Cunha et al. ('44). This suggests that it is important for one to know the previous ration fed the animals and to evaluate results obtained accordingly.

SUMMARY AND CONCLUSIONS

It has been shown that an all plant ration composed of 75.34% yellow corn, 17.50% soybean oil meal, 5.0% alfalfa meal and 2.16% minerals was inadequate for reproduction and lactation in the rat. More than 35% of the females fed the basal ration were completely sterile. Resorption and toxemia occurred frequently. Young that were born alive died within 1 or 2 days after parturition. These young attempted to nurse, but no milk could be found in their stomachs. This condition was considered to be due to lactation failure.

Previous diets were found to have a pronounced influence upon reproduction and lactation performance. There appears to be a storage of dietary essentials needed for reproduction and lactation in the rat.

The supplementation of additional alfalfa meal, 1:20 liver powder, a combination of casein (crude or acid-washed) plus choline, or fish meal improved reproduction and lactation. These data indicate that an active factor or factors are present in these supplements. Brewer's yeast, fish press water, soybean lecithin, among other materials, were variable in their supplemental effect upon reproduction and lactation.

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THE INFLUENCE OF CALORIC INTAKE ON THE GROWTH UTILIZATION OF DIETARY PROTEIN [1]

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FOUR FIGURES

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The protein efficiency method of Osborne, Mendel and Ferry ('19) has been, with modifications, one of the most extensively employed methods for the evaluation of the nutritive quality of proteins. The suggestion was made by Bosshardt et al. ('46b) that differences in the reported nutritive indices of a protein by different workers may be due, at least in part, to differences in experimental technique. The effect on the protein efficiency ratio of differences in the pretest standardization of the test animals, the duration of the test period, and the level of the test protein in the diet also were shown.

In the adult animal a restriction of the caloric intake results in an increase in urinary nitrogen excretion, indicating a decrease in the utilization of protein nitrogen. This has been related to the sparing action of carbohydrate on urinary nitrogen excretion (Lusk, '28). The data of Allison, Anderson and Seeley ('46) indicate that fat as well as carbohydrate is effective in decreasing the urinary nitrogen excretion.

[1] A preliminary report of this data was presented before the American Institute of Nutrition, Atlantic City, March 12, 1946.

It has been reported (Barnes et al., '45) that a restriction of protein intake resulting from the feeding of a diet containing a small percentage of protein or by a restriction of the entire diet through paired-feeding causes a decrease in the growth utilization of protein. It is probable that the major factor causing a decreased protein utilization under these conditions is the level of protein consumed. However, another factor that is common to both of the above conditions and may be involved is the decreased caloric intake.

The possibility exists that in protein evaluation studies involving ad libitum feeding differences between proteins may be exaggerated because of variations in the ill-defined and often overlooked appetite aspects of the diets, which result in marked differences in total food intake and thus in caloric intake. The increased growth utilization of casein when liver fractions were added to the diets, which was reported by Bosshardt et al. ('46a), may have been due, at least in part, to an increased food intake resulting from the correction of an unrecognized dietary deficiency.

This study was designed to investigate the influence of caloric intake on the growth utilization of proteins by rats and mice.

EXPERIMENTAL METHOD

Groups of eight male albino weanling rats (Sprague-Dawley strain), in individual cages, were fed isocaloric diets containing varying amounts of extracted whole egg ad libitum for 42-day periods (Barnes et al., '45). Determinations were made of weight gains and of food and protein consumptions. The typical protein utilization curve that is shown in figure 1 was obtained. The average daily energy intake per cm^2 of body surface area was calculated [2] for each group and also is shown plotted as a function of protein intake in figure 1.

In a second experiment, groups of seven male albino weanling mice (Sharp and Dohme, Swiss-Webster strain), in individual cages, were fed isocaloric diets containing varying levels of casein for 10-day periods (Bosshardt et al., '46b).

[2] B.S. $= 11.36 \times$ wt. $^{2/3}$ (Carman and Mitchell, '26).

Weight gain and food and protein intake were measured. The protein utilization and energy intake data are shown in figure 2.

In both cases, at the level of maximal protein utilization there was a maximal consumption of total calories per unit body surface area. The caloric intake per unit of body surface area decreased on either side of this point. This pattern of caloric intake and protein utilization also has been ob-

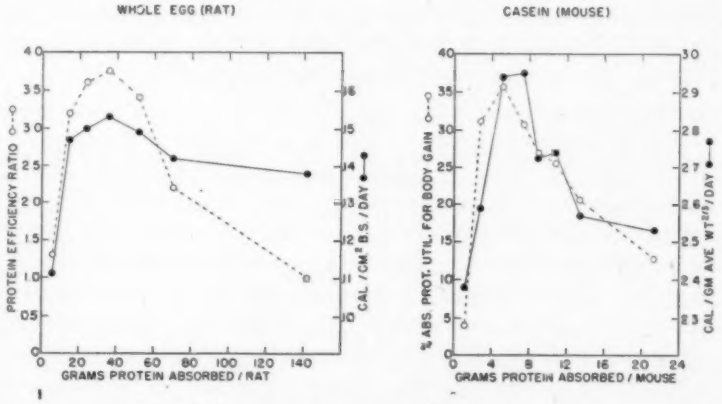

WHOLE EGG (RAT)
PROTEIN EFFICIENCY RATIO o--o
CAL / CM² B.S. / DAY
GRAMS PROTEIN ABSORBED / RAT
CASEIN (MOUSE)
% ABS. PROT. UTIL. FOR BODY GAIN o--o
CAL. / GM AVE W^2/3 / DAY
GRAMS PROTEIN ABSORBED / MOUSE

Fig. 1 The relationship between protein utilization and caloric intake with varying protein intakes. Diets containing varying levels of whole egg protein were fed to rats for 42-day periods.

Fig. 2 The relationship between protein utilization and caloric intake with varying protein intakes. Diets containing varying levels of casein were fed to mice for 10-day periods.

tained with rats that were fed isocaloric diets containing different levels of wheat gluten and two different soyflours, and with mice that were fed isocaloric diets containing different levels of extracted whole egg and wheat gluten.

It cannot be concluded from these data that optimal calories are supplied when protein utilization is maximal. However, it would appear possible that, at any level of protein intake, the growth utilization of protein may be enhanced if the nonprotein caloric intake is increased.

As a preliminary experiment to determine the influence of the caloric intake on the growth utilization of protein, five diets with varying caloric content were employed. The basic diet composition was the same as previously reported (Bosshardt et al., '46b) and contained 10% casein, but the fat: dextrin ratios were altered so that the various diets contained from 2 to 32% fat with a corresponding range of caloric values of 3.9 Cal. per gm to 5.5 Cal. per gm. These diets were fed to male weanling albino mice (Sharp and Dohme, Swiss-Webster strain), in individual cages, for 10-day periods. Weight gain and food and protein intake were recorded. The results obtained are shown in table 1.

TABLE 1

Protein efficiency ratios determined with mice in 10-day feeding periods with diets of varying caloric contents but all containing 10% casein.

FAT IN DIET	CAL./GM DIET	PROT. EFF. RATIO	N IN CARCASSES
%			%
2	3.9	1.75	2.77
12	4.4	1.77	2.88
22	5.0	2.09	2.79
27	5.2	2.20	2.72
32	5.5	2.29	2.68

As the fat level in the diet was increased with a corresponding increase in the caloric value per gm, there was a marked increase in the protein efficiency ratios which ranged from 1.75 to 2.29. However, as the fat level was increased the food intake per mouse for the 10-day period decreased from 24.8 gm to 17.4 gm with a corresponding decrease in protein intake from 2.3 gm to 1.7 gm. The total caloric intake per mouse per day, however, increased from 9.8 to 11.3. The carcass nitrogen figures indicate that in no case was there an abnormal fat deposition that would give misleading protein efficiency ratios.

Results of this nature are difficult to evaluate because of the marked differences in protein and caloric intake, neither of

which were the same for any two groups. This may help to explain some of the differences in reported nutritive indices of the same protein.

Two series of studies to investigate the influence of caloric intake on the growth utilization of protein under conditions of ad libitum feeding, but with essentially equal protein intake were set up.

Three series of diets containing a well-heated soyflour as the protein source were prepared. All diets contained 4% salt (Wesson, '32) and to each 100 gm of diet were added 0.4 mg of riboflavin, 0.4 mg of thiamine hydrochloride, 0.4 mg of pyridoxine hydrochloride, 2.5 mg of niacin, 1.1 mg of calcium pantothenate, 7.5 mg of para-aminobenzoic acid, 200 mg of choline hydrochloride, 20 mg of inositol and 0.05 mg of 2-methyl-1,4-naphthoquinone diacetate. The water soluble vitamins were mixed with a sucrose carrier and alpha tocopherol (2.5 mg) was dissolved in the fat. In addition, each animal received 2 drops of a cod liver oil twice weekly. The remainder of the diets consisted of a well heated soyflour, hydrogenated cottonseed oil,[3] sucrose, and fiber.[4] In the first series of diets the soyflour was incorporated at such a level that the protein accounted for approximately 10% of the total calories, and the fat, fiber, and sugar were varied to give a range of 2.5 to 6.6 Cal. per gm of diet. In the second series the soyflour was added so that all diets contained 10% protein by weight and the fat, fiber, and sugar were varied to give diets with a range of 2.6 to 6.5 Cal. per gm. The third series consisted of isocaloric diets with protein levels ranging from 8.1 to 13.9% by weight, or 7.7 to 13.5% of the total calories.

The different diets were fed ad libitum to groups of eight male weanling albino rats (Sprague-Dawley strain), in individual cages, for a period of 42 days. A record was kept of weight gain and of food and protein intake. From the data were selected those groups in which the average protein intake

[3] Crisco.
[4] Cellu flour.

per animal corresponded to that giving maximal utilization (Barnes et al., '45).

In figure 3 is shown the relationship between protein utilization, calculated as the protein efficiency ratio, and the average daily caloric intake per cm^2 of body surface area per day. These data indicate that there is a limit to which additional non-protein calories can enhance the growth utilization of protein. When, however, the caloric intake is reduced to below 80% of the maximal intake obtained in this experiment there is a very marked reduction in the protein utilization.

A similar experiment using mice as the test animals and casein as the protein source was carried out. The basal diet consisted of 2% corn oil,[5] 20% glucose,[6] 4% salt mixture (Hubbell, Mendel and Wakeman, '37), 2% cellulose,[4] 1% Wilson's 1:20 liver concentrate powder, and was supplemented so that each 100 gm of diet contained 4 mg of alpha tocopherol, 900 U.S.P. units of vitamin A, 180 U.S.P. units of vitamin D, 1 mg of 2-methyl-1,4-naphthoquinone diacetate, 0.8 mg of thiamine hydrochloride, 1.6 mg of riboflavin, 0.8 mg of pyridoxine hydrochloride, 4.0 mg of niacin, 4.4 mg of calcium pantothenate, 4.0 mg of para-aminobenzoic acid, 200 mg of choline chloride, and 21.6 mg of inositol. The remainder of the diets consisted of casein and hydrogenated cottonseed oil [7] the levels of which were varied so as to give three series of diets similar to those previously described.

The diets were fed ad libitum to groups of seven male weanling albino mice (Sharp and Dohme, Swiss-Webster strain) for 10-day periods. Determinations were made of weight gain and of food and protein consumption. Again the data were selected to include only those groups whose protein intakes corresponded to that giving maximal utilization (Bosshardt et al., '46b). The results are shown in figure 4.

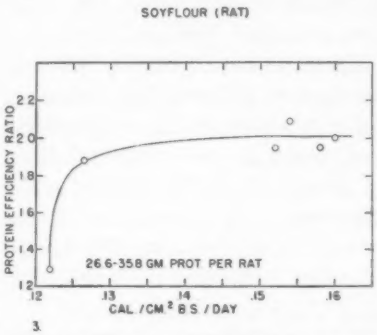
As in the previous study with rats a limit was approached above which the utilization of protein could not be increased

[5] Mazola.

[6] Cerelose.

[7] Primex.

by increasing the intake of calories. In this experiment a sharp reduction in protein utilization occurred when the caloric intake was below 95% that of the maximal intake obtained.



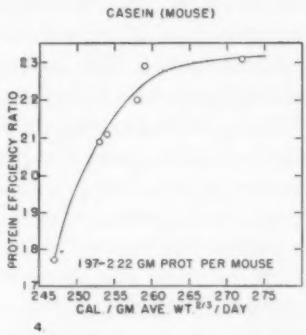


Fig. 3 The relationship between protein utilization and caloric intake with approximately equal protein intakes (see text). Diets containing a well-heated soyflour as the protein source were fed to rats for 42-day periods.

Fig. 4 The relationship between protein utilization and caloric intake with approximately equal protein intakes (see text). Diets containing casein as the protein source were fed to mice for 10-day periods.

DISCUSSION

Larson and Chaikoff ('37) have indicated that, under proper conditions, the feeding of additional carbohydrate to adult dogs that were considered to be in a "normal" state of nutrition reduced the urinary nitrogen excretion, indicating a sparing effect of carbohydrate on protein metabolism. The diet maintaining the "normal" state of nutrition supplied 60 Cal. per kg of body weight per day. Melnick and Cowgill ('37) and Allison and Anderson ('45) have shown that the minimal protein intake required to maintain nitrogen balance in adult dogs varied with individual dogs. These results were obtained by providing all test animals with an equal caloric intake. Allison and Anderson ('45) observed in one group of three dogs that an increase from 80 to 100 Cal. per kg of body weight per day resulted in a decreased urinary nitrogen excretion with a corresponding decrease in the amount of protein necessary to maintain nitrogen balance.

Allison, Anderson and Seeley ('46) have defined the nitrogen balance index as the rate of change of nitrogen balance with respect to absorbed nitrogen. The nitrogen balance index is equal to the biological value of Thomas ('09) (the proportion of absorbed nitrogen retained by the body) if it can be assumed that nitrogen excretion on a protein-free diet is "endogenous" nitrogen. The data of Allison and Anderson ('45) indicate that within limits a variation of the non-protein caloric intake alters the urinary nitrogen excretion without a change in the nitrogen balance index. If the feeding of additional non-protein calories decreases the urinary nitrogen excretion without altering the nitrogen balance index, the effect must be one of decreasing the "endogenous" nitrogen metabolism of the animal.

More recent results (Allison, Anderson and Seeley, '46) have indicated that the nitrogen balance index in adult dogs is not affected until the caloric intake is reduced to below 50% of that considered by them to be adequate for optimal protein utilization. A reduction of the caloric intake to 25% of that adequate for optimal protein utilization resulted in a marked decrease in the nitrogen balance index. At no level of protein intake was positive nitrogen balance obtained at the 25% caloric intake level.

The results of Allison and co-workers would suggest that when the non-protein caloric intake is reduced systematically two types of response are encountered. In the first type, which is observed when the caloric restriction is relatively small, the nitrogen balance index remains essentially constant, although the "endogenous" metabolism changes. In the second type, where a more severe caloric restriction is imposed, there is a marked decrease in the nitrogen balance index.

The data obtained in the present study may be considered to correspond to the first type of response since the animals were in positive nitrogen balance and changes in caloric intake were small. As is shown in figures 3 and 4, with essentially constant protein consumption decreases in caloric intake resulted in decreases in protein efficiency ratios (gm gain in

body weight per gm of protein consumed). There was, however, a range of caloric intake that maintained maximal protein utilization. Increasing the caloric consumption beyond this was without effect on the protein efficiency ratio.

In the growing animal an increase in "endogenous" excretion will result in a smaller proportion of the absorbed protein that is used in new tissue formation. This in turn will result in a decreased protein efficiency ratio. However, since growing animals are in positive nitrogen balance, the decrease noted in the protein efficiency ratio when the caloric intake is decreased most probably is not a reflection of a decrease in the nitrogen balance index but rather of an increase in "endogenous" metabolism.

These studies have made possible the estimation of caloric intake per unit body size that is necessary for the optimal growth utilization of soyflour protein and casein by rats and mice, respectively. There is no evidence that these values can be applied to other dietary proteins. However, it is of considerable importance to point out that with poor proteins such as wheat gluten supplied in ordinary isocaloric diets, caloric intake never reaches the plateau levels shown in figures 3 and 4 regardless of the amount of protein in the diet. This may mean that voluntary food intake is so severely inhibited by poor proteins that suboptimal caloric intake results. It is well recognized that poor proteins give lower nutritive value indices in growing animals than in adult maintenance studies (Barnes et al., '46). Part of the discrepancy between the two types of evaluation may be due to self-imposed caloric deficiencies that cause an exaggerated decrease in the utilization of the low quality proteins.

It has been pointed out previously (Bosshardt et al., '46a, '46b) that various alterations in experimental technique may cause differences in the nutritive indices of proteins. This report indicates the necessity of the control of the non-protein caloric intake if optimal values are to be obtained. Diets may be compounded in such a manner that, even though the animals are eating ad libitum, a self-imposed caloric restriction that

is sufficient to result in suboptimal protein utilization may occur.

SUMMARY

Caloric intake and protein utilization have been compared in growing rats and mice that were eating ad libitum.

With each protein source it was found that the level of intake exhibiting maximal protein utilization coincided with a maximal caloric intake per unit of body surface area.

At a given level of protein intake, changes in caloric consumption often resulted in changes in the apparent utilization of protein.

It is important in determinations of protein quality to maintain the non-protein caloric intake at a level at which optimal values will be obtained.

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SPECIFIC DYNAMIC ACTION OF DIETS HIGH IN CARBOHYDRATE OR FAT

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It is a well established fact that the specific dynamic action (S.D.A.) of a mixture of foodstuffs is less than the total S.D.A. of the protein, carbohydrate and fat of which it is composed. One is, however, not certain whether carbohydrate or fat is more effective in lowering the S.D.A. of protein. Ring ('42) measured the S.D.A. of individual foods and of mixtures after these had been given to fasting rats. He found that carbohydrate and protein summated much less completely than fat and protein. On the other hand, Forbes and Swift ('44), using their usual method of comparing the metabolism on a basal diet with that when various foods and mixtures were added to this basal diet, found fat was more effective in reducing the S.D.A. of protein than was carbohydrate. The following figures show how much less than completely additive various mixtures of foodstuffs are according to these two laboratories: (a) protein and carbohydrate, 40% less (Ring) compared with 12.5% less (Forbes and Swift); (b) protein and fat or fatty acid, 15% (Ring), and 54% (Forbes and Swift); and (c) carbohydrate and fat or fatty acid, 60% (Ring) compared with 35% (Forbes and Swift).

These differences in results are certainly due in part to not using the same foods, but we believe that more important than this is the difference in methods of measurement. Forbes and Swift have superimposed protein on a diet high in carbohydrate. In this case there should be little tendency for the

protein to be converted to carbohydrate and the addition of more carbohydrate should not lower the S.D.A. of protein very greatly. On the other hand, when an animal with an R.Q. of 0.72, as in Ring's work, is given protein, this is probably converted in considerable part to carbohydrate with a large waste of energy. Carbohydrate given with protein should reduce this tendency. Furthermore, fat given to a rat with a low R.Q. and burning much fat already would probably not effectively reduce the S.D.A. of protein.

The question which arises from these observations is, "Does a diet high in fat or one high in carbohydrate have the lower S.D.A.?" According to Ring's observations, the S.D.A. of a diet high in fat should be slightly greater than that of one high in carbohydrate. On the other hand, Forbes and Swift state, "While there is a question as to the applicability of results obtained with one species to the nutrition of another species, the results of this study suggest, at face value, that it is not necessary to diminish the protein content of the hot weather human diet in order to have a low heat increment, since this purpose can be accomplished by equicaloric substitution of fat for carbohydrate.

"It thus appears that there is a physiological economy in the high fat content of the diet of working people of some tropical countries — notably Brazil."

METHOD

In order to test these opinions, we have prepared two diets each with the same amount of protein, minerals and vitamins. One diet was high in fat and the other high in carbohydrate. The composition of each is shown in table 1.

During a previous study, we had been using small rats (about 50 gm) and in order to reduce control measurements we have continued this practice. These rats were kept on one of the above diets for at least 1 week prior to the first metabolic measurements. The Haldane principle was used for determining the gaseous metabolism, and protein was assumed to be burned in the proportion ingested. The respira-

tion chambers were kept at 30°C. The control measurements were made over a period of 7 hours during which no food was allowed. The next day the rats ate a weighed amount of food, and the metabolism was subsequently determined, this time for a period of 8 hours. Only quiet periods were used in calculations though it was found that the figures for S.D.A. were almost the same if one subtracted the total metabolism of the control period from the total after the ingestion of food. In other words, the activity of the rats was about the same whether fed or not.

TABLE 1

Composition of diets.

	HIGH CARBOHYDRATE DIET	HIGH FAT DIET
Casein (vitamin free) — gm	214	214
Corn oil — gm	40	261
Cod liver oil — gm	20	20
Sucrose — gm	626	118.5
Yeast (autoclaved) — gm	60	60
Salt mixture no. 1 (U.S.P. XII p. 637) — gm	40	40
Succinylsulfathiazole — gm	10	10
Thiamine [1] — mg	10	10
Ca pantothenate [1] — mg	20	20
Choline [1] — mg	1,000	1,000
Inositol [1] — mg	1,000	1,000

[1] Kindly supplied by Merck and Co., Inc., Rahway, N. J.

It is possible that 8 hours is not long enough to obtain the entire S.D.A. This, however, is not important for these results since the S.D.A. of a high fat diet should last longer than that for a high carbohydrate diet, and the true value of S.D.A. for the fat will, if anything, be more reduced than that for carbohydrate. Thus the evidence will be weighed in favor of a low S.D.A. for the fat diet.

RESULTS

In table 2 are shown the S.D.A.s of animals maintained on high fat or high carbohydrate diets. The S.D.A.s appear to be

about the same for each group. Though the mean figures show the S.D.A. for the fat diet ($7.10 \pm 0.60\%$) to be higher than that for the carbohydrate diet ($6.65 \pm 0.33\%$), the standard deviations indicate that the true mean for the fat diet may lie very slightly below that for carbohydrate.

The intake of food just prior to these measurements of S.D.A. was kept approximately the same for each group — 23.1 Cal. per 100 gm of rat for those receiving the carbohydrate diet, and 22.0 Cal. for those on the fat diet. The slightly smaller food intake on the fat diet will tend to keep the S.D.A. low on this regime, since Forbes, Kriss and Miller ('34) have shown that the S.D.A. increases with increase in food intake.

TABLE 2

The respiratory quotients and the specific dynamic effect of a high carbohydrate and a high fat diet.

HIGH CARBOHYDRATE DIET			HIGH FAT DIET		
Respiratory quotients		S.D.A.[1]	Respiratory quotients		S.D.A.[1]
Fasting	Fed		Fasting	Fed	
(0.640)	0.914	3.47	0.753	0.730	5.98
0.715	0.862	7.71	0.724	0.768	14.18
0.804	0.983	5.87	0.754	0.779	6.21
0.756	0.910	6.56	0.722	0.713	3.97
0.827	0.941	4.76	0.743	0.733	6.35
0.787	0.863	8.91	0.731	0.722	7.70
	0.960	6.11	0.709	0.748	4.93
0.846	0.950	8.41	0.831	0.723	8.78
0.965	1.044	6.52	0.716	0.733	6.93
0.787	0.969	8.92	0.752	0.742	6.01
0.846	0.849	6.27			
0.803	0.883	8.44			
0.777	0.936	4.56			
Av. 0.810	0.928	6.65	0.743	0.739	7.10
±0.015	±0.010	±0.33	±0.007	±0.004	±0.60

[1] Calculated as per cent of calories in food consumed.

The measurement of metabolism on the high fat and high carbohydrate diets gave quantitatively the same results both during control determination and after feeding when calcu-

lated per 100 gm of rat per hour. For the high carbohydrate feeding, the control and "after feeding" values, respectively, were 0.812 Cal. and 0.998 Cal.; for the high fat feeding, the corresponding values were 0.810 and 1.002 Cal.

The respiratory quotients were, of course, high with carbohydrate and low with fat (see table 2).

If the S.D.A. for these two types of diet were markedly different, it might well be reflected in food intake. The food ingested by groups of four animals has been carefully measured over a period of 2 or 3 weeks. Subtracting from these results the figures for the additional energy stored in the bodies of these growing rats (see Wynn and Haldi, '44), we obtained the following results, given in Cal. per 100 gm per day: for high carbohydrate diet 26.79, 28.19, 31.45 and 32.20, with an average of 29.66; for high fat diet 29.77, 30.70, 31.27 and 31.99, with an average of 30.93.

These figures do not include any correction for loss of energy in the feces or urine. They do include the energy required for normal activity. The observations suggest that as much energy is required when ingesting a high fat diet as when living on a high carbohydrate regime. All the above observations are in accord with previous work of Ring ('42). They show that when fat is substituted for carbohydrate, the S.D.A. of the diet is not reduced (see Forbes and Swift, '44).

CONCLUSIONS

1. The S.D.A. on a high carbohydrate diet ($6.65 \pm 0.33\%$ of energy in food ingested) is about the same as that of a high fat diet ($7.10 - 0.60\%$) (see table 2) containing the same amount of protein.

2. Respiratory metabolism for quiet periods during the 8 hours subsequent to the ingestion of a meal high in carbohydrate averaged 0.998 Cal. per 100 gm per hour, and is almost the same as after ingesting an isocaloric meal high in fat — 1.002 Cal. per 100 gm per hour.

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EFFECT OF PROTEINS LOW IN TRYPTOPHANE ON GROWTH OF CHICKENS AND ON LAYING HENS RECEIVING NICOTINIC ACID-LOW RATIONS [1]

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ONE FIGURE

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That young chickens require a dietary source of nicotinic acid, when fed certain purified diets, was demonstrated by Briggs, Mills, Elvehjem and Hart ('42). Briggs ('45) reported that the addition of 10% of gelatin to a highly purified ration caused an increase in the nicotinic acid requirement of chicks and that tryptophane acted similarly to nicotinic acid in preventing the deficiency.

Previously, it had been found by Krehl, Teply, Sarma and Elvehjem ('45), working with rats, that the inclusion of tryptophane or nicotinic acid in certain low casein diets prevented a deficiency produced by the feeding of corn. Recent reports by Krehl, Sarma, Teply and Elvehjem ('46) and Krehl, Sarma and Elvehjem ('46) showed that the growth-

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inhibiting effect of corn was related to the nature of its protein and that similar inhibiting effects could be produced with certain non-corn rations.

In addition to results secured with the chicken and rat, Chick, Macrae, Martin and Martin ('38) and Wintrobe, Stein, Follis and Humphreys ('45) in studies with the pig, and Axelrod, Morgan and Lepkovsky ('45) with the dog, have also noted a relationship between the protein content of the ration and the nicotinic acid requirement. The relationship of the tryptophane content of the protein to the nicotinic acid requirement of these animals was not established in these cases.

Studies with various animals have established the fact that the presence of high amounts of corn, or products derived from corn, in a diet may increase the nicotinic acid requirement. In work with chickens, Sarma and Elvehjem ('46) reported that the addition of large amounts of corn grits to a nicotinic acid-low ration had a deleterious effect on growth, which was counteracted by the feeding of nicotinic acid. Scott, Singsen and Matterson ('46) also reported that chicks receiving a simplified diet containing corn and gelatin required nicotinic acid to prevent perosis and for growth. In work with rats, Krehl, Teply and Elvehjem ('45a) produced a nicotinic acid deficiency by feeding high amounts of corn. These results with the rat have been confirmed by Dann ('46a). Handler ('43) suggested that the presence of corn meal in rations for dogs may be a causative factor in pellagra. Krehl, Teply and Elvehjem ('45b) reported that when corn grits were added to a synthetic ration, the nicotinic acid requirement of the dog was increased. In work with swine, Davis, Freeman and Madsen ('40) noted necrotic enteritis, due to a deficiency of nicotinic acid, especially prevalent in pigs fed diets rich in corn in contrast with other grains. It has been known for a number of years that pellagra in humans was associated with large amounts of corn or corn products in the diet. The work with humans has been reviewed (Anonymous, '45).

The results presented in this paper extend the former observations on the relationship of proteins low in tryptophane

to the nicotinic acid requirement and, in addition, offer evidence for the need of nicotinic acid in the diet of laying hens. Studies with a practical ration are also given. A convenient method of assay with the chick for the nature of the inhibitory action of protein is presented.

EXPERIMENTAL

Day-old New Hampshire chicks, of mixed sexes, were divided into uniform groups of six and raised in electrically heated batteries with wire floors. Feed and water were given ad libitum. Weighings and other observations were made weekly and the experiments conducted for a period of 4 weeks.

The basal ration, 108GN, low in nicotinic acid and low in a source of arginine and glycine, consisted (in %) of Cerelose 68.4, crude casein 18, Liver Fraction L 3, soybean oil 4, Salts 1M (Briggs, '46) 6, l-cystine 0.3. Each 100 gm also contained the following amounts of vitamins (in mg): thiamine HCl 0.4, riboflavin 0.8, Ca pantothenate 2.0, choline HCl 200, pyridoxine HCl 0.6, biotin 0.02, i-inositol 100, p-aminobenzoic acid 0.2, 2-methyl-1,4-naphthoquinone 0.1, and alpha-tocopherol 0.5. Vitamin A and vitamin D_3 (1200 U.S.P. units and 170 A.O.A.C. units, respectively) were fed by dropper weekly. This ration is similar to that used by Briggs et al. ('42) which contained 0.3 mg of nicotinic acid per 100 gm. Substitutions in the ration were made at the expense of the carbohydrate.

Ration 2 was designed to be similar to certain practical-type rations (containing no animal protein supplement) and also to be fairly low in nicotinic acid and high in corn. It was composed as follows: ground yellow corn 50, ground oats 10, wheat middlings 5, wheat bran 5, soybean oil meal 13, corn gluten meal 13, oyster shell flour 2, steamed bone meal 1.4, sodium chloride 0.5, manganese sulfate 0.012, vitamin D_3 (Delsterol) 200 A.O.A.C. units per 100 gm, and riboflavin 0.4 mg per 100 gm. Substitutions to this ration were made at the expense of corn. Ration 2 was calculated to contain approximately 4.5 mg of nicotinic acid per 100 gm by use of the nico-

tinic acid values in feedstuffs as given by Hale, Davis and Baldwin ('42).

The studies with laying hens (New Hampshire pullets) were conducted in conventional-type steel laying batteries. Artificial inseminations were performed twice weekly with the pooled semen of three New Hampshire roosters. Eggs were collected daily. Weekly settings of eggs were made and hatchability recorded.

RESULTS

In table 1 a summary is given of a number of experiments dealing with the effect of the addition of various levels of gelatin to ration 108GN, with and without nicotinic acid, on mortality, growth, blacktongue, perosis, and efficiency of feed utilization. Results obtained with the presence of corn are also given.

As the level of gelatin (which supplies sufficient arginine and glycine for the chicken when fed at a level of 10%) was increased in the ration above the 5% level, an actual depression in growth rate occurred (compare groups 1, 3, 6, and 13). Growth depression was accompanied by increased mortality, perosis, and blacktongue. Other deficiency symptoms such as poor feathering, diarrhea, excessive amount of mucous in the mouth, unkempt appearance of the head, and accumulation of feed under the tongue ("food canker") were noted in a majority of the chicks. Some of these latter "symptoms" may have been due to the physical character of the ration (i.e., the presence of gelatin causes the ration to become sticky when moistened). The growth depression was most marked with the highest levels of gelatin fed but was overcome by the addition of nicotinic acid or tryptophane to the ration. Lower amounts of tryptophane were not as effective (Briggs, '45).

It is evident, as would be expected, that sufficient arginine and glycine (supplied by gelatin) must be present before nicotinic acid can give maximum growth (compare group 2 with groups 17 and 18). The lack of perosis with 20% gelatin alone may be attributed to the very poor growth rate. Higher

TABLE 1

Effect of gelatin and corn on growth, blacktongue, perosis, efficiency, and nicotinic acid requirement of chicks.

GROUP	SUPPLEMENT TO BASAL RATION 108GN (WITH NO GELATIN)	NO. CHICKS STARTED	NO. DEAD AT 4 WKS.	AVERAGE WEIGHT AT 4 WKS.	PER CENT WITH BLACK-TONGUE	PER CENT WITH PEROSIS	EFFICIENCY [1]
				gm			
1	None	12	1	88	8	25	0.350
2	5 mg nicotinic acid/100 gm	12	2	101	0	0	0.275
3	5% gelatin	35	1	247	43	20	0.505
4	5% gelatin + 1 mg nicotinic acid/100 gm	6	0	283	0	17	0.505
5	5% gelatin + 5 mg nicotinic acid/100 gm	18	0	298	0	0	0.521
6	10% gelatin	72	12	138	89	64	0.358
7	10% gelatin + 1 mg nicotinic acid/100 gm	6	0	237	67	50	0.546
8	10% gelatin + 2 mg nicotinic acid/100 gm	12	1	313	0	42	0.526
9	10% gelatin + 3 mg nicotinic acid/100 gm	6	0	299	0	67	0.547
10	10% gelatin + 5 mg nicotinic acid/100 gm	65	1	328	0	15	0.565
11	10% gelatin + 10 mg nicotinic acid/100 gm	12	1	333	0	8	0.512
12	10% gelatin + 0.2% dl-tryptophane	12	0	343	0	25	0.588
13	20% gelatin	6	5	75	83	0	0.148
14	20% gelatin + 5 mg nicotinic acid/100 gm	12	1	333	33	50	0.581
15	10% bone ossein	6	2	116	83	17	0.296
16	10% bone ossein + 5 mg nicotinic acid/100 gm	12	0	311	0	9	0.600
17	0.8% l (+) arginine + 2.5% glycine	30	1	258	37	30	0.517
18	As group 17 + 5 mg nicotinic acid/100 gm	6	0	296	0	0	0.445
19	As group 17 + 1% alanine	6	0	208	0	83	0.534
	Following groups contain corn in place of Cerelose:						
20	10% gelatin	12	2	130	92	75	0.370
21	10% gelatin + 1 mg nicotinic acid/100 gm	6	0	248	50	50	0.530
22	10% gelatin + 2 mg nicotinic acid/100 gm	6	0	359	0	50	0.591
23	10% gelatin + 5 mg nicotinic acid/100 gm	6	0	373	0	33	0.647
24	10% gelatin + 10 mg nicotinic acid/100 gm	6	0	364	0	0	0.619
25	10% gelatin + 0.2% dl-tryptophane	12	0	306	0	66	0.599

[1] $\frac{\text{Total weight gained}}{\text{Total feed consumed}}$

levels of gelatin caused the ration to become too gummy when eaten by the chicks.

Bone ossein, from which gelatin is derived, caused a similar depression of growth in the absence of nicotinic acid.

When an amount of arginine and glycine equal to that found in 10% of gelatin was fed, near maximum growth occurred. This was improved further, however, by the addition of nicotinic acid to the ration. The addition of alanine to the combination of arginine and glycine definitely depressed the growth rate and gave a high incidence of perosis (group 19, table 1).

The feeding of large amounts of corn with gelatin gave results similar to those with gelatin alone. Apparently the action of the corn and gelatin was not additive with 10% of gelatin in the ration. The addition of nicotinic acid to the corn rations resulted in an extremely fast rate of growth for this species (appreciably greater growth than when nicotinic acid was added to the Cerelose rations). This suggests that corn contains an unidentified growth-promoting factor or factors not present in the basal ration. Tryptophane appeared to be somewhat less effective when added to the corn diet than when added to the Cerelose diet (compare groups 12 and 25).

It is evident, from data presented in table 1, that the amount of nicotinic acid which was necessary for maximum growth varied with the amount of gelatin or tryptophane fed. For example, 1 mg of nicotinic acid resulted in better growth and less blacktongue and perosis with the presence of 5% of gelatin than with 10% of gelatin (compare group 4 with group 7). No nicotinic acid was needed for growth when ample tryptophane was present although some perosis did occur. Thus, the chicken may require anywhere from little or no nicotinic acid in a diet to as high as at least 5 to 10 mg per 100 gm of ration. The amount required by New Hampshire chicks on this ration (a minimum of approximately 5 mg per 100 gm) appears to be considerably higher than the amount set by Briggs et al. ('42) for the White Leghorn chick (1.8 mg per 100 gm of a similar ration containing 10% of gelatin) and

is similar to the amount required by the turkey under such a regime (Briggs, '46).

Method of assay of factors responsible for depressing action

Because fairly good growth was obtained by the inclusion of only 5% of gelatin in the ration whereas higher levels of gelatin caused a marked depression in growth, a means of assay could be devised for the routine determination of the depressing action of various supplements. The assay was based upon the depressing effect of the supplements when added to the basal ration 108GN plus 5% of gelatin.

The results of such an assay are summarized in table 2. The addition of high levels of gelatin (groups 2 and 3) and ossein (group 4) to the ration had severe depressing effects. (This character of these proteins was later utilized in producing a nicotinic acid deficiency in chicks receiving practical rations and in hens). Casein and soybean oil meal, as well as oats and wheat (groups 5 to 8), had no depressing effect.

By comparing the activities of corn, corn gluten meal, and zein (groups 9 to 13), it is evident that the depressing action of corn is due to its protein content, as Krehl, Sarma and Elvehjem ('46) have likewise reported. The depressing action of corn and corn gluten meal was again prevented by the presence of nicotinic acid. It is very interesting to note that zein was only about one-third as depressing as gelatin (see groups 2, 3, and 11). This indicated that more is probably involved in this problem than just the feeding of a tryptophane-low protein itself.

Since such a marked depression was obtained with gelatin, and since it is known that certain of the amino acids in gelatin have a detrimental effect when added to certain rations (see review by Hier, Graham and Klein, '44; and also Patton, '39), several amino acids were fed in combination with 5% of gelatin (groups 14 to 20, table 2). Again alanine had a definite depressing action as did the combination of arginine and glycine. The depressing action of these amino acids was ap-

parently due to an increased nicotinic acid requirement, as evidenced by an increase in the severity of deficiency symptoms. Also, the condition is overcome by the addition of nicotinic acid to the diet containing the amino acids (unpublished data). This work in itself does not yet explain all of the depressing action of gelatin. It indicates, however, that at least part of the effect of gelatin can be accounted for by its amino acid content.

TABLE 2

Assay for depressing action of various materials and studies with several amino acids.

GROUP	SUPPLEMENT TO BASAL DIET 108GN CONTAINING 5% OF GELATIN	NO. CHICKS STARTED	NO. DEAD AT 4 WKS.	AVERAGE WEIGHT AT 4 WKS.	PER CENT WITH BLACK-TONGUE	PER CENT WITH PEROSIS	EFFICIENCY
				gm			
1	None	35	1	247	43	20	0.505
2	5% gelatin	72	12	138	89	64	0.358
3	15% gelatin	6	5	75	83	0	0.148
4	15% bone ossein	6	5	57	100	0	....
5	15% casein	6	0	284	0	33	0.603
6	15% soybean oil meal	6	0	335	33	17	0.568
7	25% oats	6	0	299	83	67	0.566
8	25% wheat	6	0	272	50	33	0.492
9	25% yellow corn	12	0	208	75	33	0.524
10	25% corn gluten meal (44% protein)	18	1	176	94	61	0.473
11	15% zein	6	0	155	83	67	0.452
12	25% yellow corn + 5 mg nicotinic acid/100 gm	6	0	295	0	0	0.559
13	25% corn gluten meal + 5 mg nicotinic acid/100 gm	24	0	355	0	0	0.608
14	0.5% alanine	6	0	231	67	17	0.475
15	2% alanine	6	0	191	83	17	0.507
16	1.25% glycine	6	0	216	50	33	0.439
17	0.5% alanine + 1.25% glycine	6	0	237	83	50	0.483
18	0.4% l (+) arginine	12	0	209	83	42	0.475
19	0.4% l (+) arginine + 1.25% glycine	12	1	184	67	33	0.478
20	0.4% l (+) arginine + 0.5% alanine	6	1	195	83	17	0.448

Studies with a practical ration

Because the feeding of gelatin and bone ossein had greatly increased the need for nicotinic acid, these materials were added to ration 2 with and without the presence of added nicotinic acid (see table 3) and fed to day-old chicks. In the absence of nicotinic acid, gelatin and ossein caused a marked

TABLE 3

Summary of studies with a practical-type ration. (New Hampshire chicks.)

GROUP	SUPPLEMENT TO RATION 2	NO. CHICKS STARTED	NO. DEAD AT 4 WKS.	AVERAGE WEIGHT AT 4 WKS.	PER CENT WITH BLACK-TONGUE	PER CENT WITH PEROSIS	EFFICIENCY
				gm			
1	None	38	1	183	0	16	0.380
2	20 mg nicotinic acid/100 gm	6	0	200	0	0	0.385
3	10% bone ossein	32	6	126	50	34	0.358
4	10% bone ossein + 20 mg nicotinic acid/100 gm	6	0	177	0	17	....
5	10% gelatin	12	2	130	17	8	0.265
6	10% gelatin + 20 mg nicotinic acid/100 gm	6	1	181	0	17	0.264
7	10% gelatin + 0.2% dl-tryptophane	6	0	153	0	33	0.338
8	10% tankage	6	0	206	0	67	0.398
9	10% tankage + 20 mg nicotinic acid/100 gm	6	0	245	0	33	0.413
10	10% crude casein	22	0	231	0	14	0.445

retardation of growth and increased symptoms of nicotinic acid deficiency (see groups 3 and 5, table 3). This poor growth was counteracted by the feeding of nicotinic acid. The results with tankage, which contains considerable gelatin-like proteins, indicated that this feedstuff could be supplemented to advantage with nicotinic acid in such rations. The good growth obtained in groups 9 and 10 with tankage and nico-

tinic acid and with casein showed that the protein quality in ration 2 was poor or that certain other nutrients were lacking.

Studies with laying hens receiving purified diets

In preliminary work ration 104N,[2] similar to the basal purified ration 108GN but with 5% of gelatin, was fed to four laying New Hampshire pullets. Four additional pullets were fed ration 104N supplemented with 20 mg of nicotinic acid per 100 gm of ration. No differences in egg production, body weight, or hatchability were noted after a 3 months' feeding trial.

When it was found in the work with chicks that greater amounts of gelatin or bone ossein than 5% were necessary to give a depressing action in the absence of nicotinic acid, eight more New Hampshire pullets were placed on experiment and were divided into two groups of four hens each, with and without nicotinic acid. They were fed a basal ration similar to ration 104N but with a total of 20% of ossein. Corn starch was used instead of Cerelose. Ossein was chosen because it did not cause the ration to be sticky when moistened, as did gelatin.

The four hens given the basal ration without nicotinic acid (group A) began to lose weight rapidly and showed a loss of egg production as well as a marked drop in hatchability (see table 4 and fig. 1). Many of the eggs were soft-shelled. The hens in group B, which were given 50 mg of nicotinic acid per 100 gm of ration, maintained their weight well and had normal egg production and hatchability.

After a period of 6 weeks, when all the hens in the deficient group had lost considerable weight and one had become quite moribund, the rations were reversed. Within a few days hens in group A began to gain weight and continued to gain throughout the experiment, with the exception of the moribund hen which died. Egg production and hatchability increased

[2] Ration 104N contained only 15% of casein, 5% of gelatin, and an increased amount of vitamins A and D by capsule (7 drops) weekly. Oyster shell was fed ad libitum.

TABLE 4

The effect of a nicotinic acid deficiency in laying hens on egg production and hatchability.

GROUP A NO NICOTINIC ACID			GROUP B WITH NICOTINIC ACID		
Weeks on experimental diets	Eggs/hen/day	Per cent hatchability	Weeks on experimental diets	Eggs/hen/day	Per cent hatchability
1	0.392	100	1	0.678	72.7
2	0.285	50	2	0.500	55.6
3	0.250	0	3	0.642	90.0
4	0.211	0	4	0.714	87.5
5	0.285	0	5	0.678	72.7
6	0.107	0	6	0.642	81.8
		Diets reversed			
7	0.035	0	7	0.642	62.5
8	0.211	80	8	0.535	67.0
9	0.250	67	9	0.500	0.0
10	0.240	100	10	0.392	28.6
11	0.190	100	11	0.250	33.3
12	0.238	...	12	0.250	...
13	0.333	...	13	0.285	...

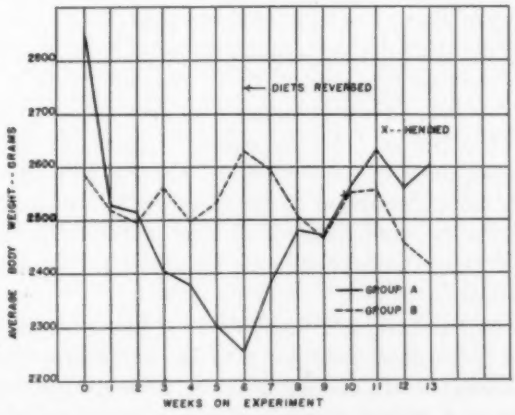

Fig. 1 Growth results with hens receiving high amounts of ossein. Group A, no nicotinic acid (first 6 weeks); Group B, with nicotinic acid (first 6 weeks). Diets reversed at 6 weeks.

markedly. The former positive control birds (group B), which were now given the deficient ration, lost weight and had a marked drop in egg production. The experiments were discontinued when ground ossein no longer was available.

Although a relatively small number of hens was used, the results showed quite conclusively, and for the first time, that hens under certain conditions require nicotinic acid in the diet for proper weight maintenance, egg production, and hatchability. No "blacktongue" symptoms were noted in the hens.

DISCUSSION

The reason for the depressing effect of gelatin and similar proteins low in tryptophane, and its counteraction with nicotinic acid, has not been accurately determined as yet. The data presented in this paper indicate that certain of the amino acids present in the proteins are at least partially responsible for this detrimental action. The amino acids arginine, glycine, and alanine account for some of the depressing action of gelatin. It is possible that nicotinic acid is necessary for the metabolism of excess amino groups. This view is strengthened by the work of Dann ('46b) who found that 2% of nicotinamide in a practical diet depressed the growth rate of chicks considerably whereas this same level of nicotinic acid did not.

Woolley ('46), in work with mice, has recently published evidence of a potent "pellagragenic" agent, in corn, which has been considerably concentrated. It is soluble in a mixture of chloroform and sodium hydroxide. No direct evidence for the existence or non-existence of such a factor in gelatin or corn, other than the amino acids themselves, has been found in our studies.

No reason has been demonstrated in this work as to why it is possible to replace nicotinic acid in the ration with tryptophane. However, the recent work of Rosen, Huff and Perlzweig ('46) and Singal, Briggs, Sydenstricker and Littlejohn ('46) with rats indicates that tryptophane may be an important precursor of nicotinic acid synthesis. It is well known that tryptophane can give rise to kynurenic acid, containing

a pyridine ring, which indicates that such synthesis may be possible. In our studies, the addition of sufficient nicotinic acid to the basal ration was always as effective as feeding much larger amounts of tryptophane. Thus, we have no evidence that tryptophane is being synthesized in the intestinal tract due to the presence of nicotinic acid, as suggested by Krehl, Sarma, Teply and Elvehjem ('46). Also, we feel, in the case of the chick at least, that most of the synthesis of nicotinic acid is within the body tissues rather than in the intestinal tract. Briggs, Luckey, Teply, Elvehjem and Hart ('43) showed that only a small portion of the chick's nicotinic acid requirement on such diets is synthesized within the intestinal tract. The fact that the depressing action is obtained on two widely different rations is further evidence of synthesis within the tissues of the chicken.

Since the tryptophane requirement of chickens is dependent upon the amount of nicotinic acid in the ration (and vice versa), it is difficult to attempt to set the dietary requirements for nicotinic acid or tryptophane unless the composition of the diet is considered. It is clear from our results that the amount of tryptophane supplied by 18% of casein (approximately 0.22 gm per 100 gm according to Kratzer, '44) is sufficient when ample nicotinic acid is present. The requirement of tryptophane for chicks, as determined by Grau and Almquist ('44), was 0.25% of the diet.

Berry, Carrick, Roberts and Hauge ('43), and Gericke ('44), have given evidence that certain practical poultry rations may be deficient in nicotinic acid. Richardson, Hogan and Kempster ('45) noted an actual increase in the incidence of perosis when nicotinic acid was omitted from a practial ration "diluted" with a synthetic ration. Briggs et al. ('43) previously had observed perosis in nicotinic acid-deficient chicks.

The results given in this study show that a nicotinic acid deficiency may occur in chickens under practical conditions if the protein (amino acid) content is unbalanced. It is interesting to note in this respect that Prange, Hauge and Carrick

('27) reported that gelatin caused a depression in growth rates of chickens when added to a practical ration containing large amounts of corn and meat meal. Also, McGinnis ('46) and Lucas, Norris and Heuser ('46), working with turkeys and chickens, respectively, have shown that gelatin increases the incidence of perosis when added to certain diets. Jukes ('41) reported that gelatin, when added to certain rations, increased the incidence of perosis. Perosis was prevented entirely by the addition of choline, however. It is interesting to note further that Massengale ('29) reported that high levels of meat scraps (which contain considerable gelatin) in rations for poultry resulted in poor growth and in a condition similar to perosis. The addition of yeast or milk corrected the condition. It is quite possible that an unrecognized nicotinic acid deficiency was produced in all these cases although nicotinic acid was not considered. The diets appear to have less nicotinic acid in them than the minimum amount necessary under extreme conditions.

Considerable work has been done with other animals in respect to an inhibitory action of gelatin on growth. Again it is possible that a nicotinic acid deficiency was involved. Hier et al. ('44) have noted an inhibitory action of gelatin in certain rations for rats, as well as Jackson, Sommer and Rose ('28) who reviewed the older literature on this subject. References to work with humans in this respect may be found in a report by Robscheit-Robbins, Miller and Whipple ('44).

It is well known that an excessive amount of corn in rations for chickens may cause growth depression or other difficulties. Taylor, Lerner and De Ome ('44) have noted an increase in laying hen mortality due to the presence of high amounts of corn in the ration. These authors reviewed previous work with chickens in this respect. In light of the present study and the recent work by Sarma and Elvehjem ('46) on the influence of corn on the nicotinic acid requirement of chickens, an adequate supply of nicotinic acid in the diet in future work of this type would be important.

SUMMARY

The nicotinic acid requirement of chickens was influenced by the protein content of the ration. The presence of an excessive amount of a protein low in tryptophane (e.g., gelatin or zein) in purified, nicotinic acid-low rations depressed the growth rate of young chickens and increased the severity of nicotinic acid deficiency symptoms (blacktongue, perosis, etc.). Gelatin was about three times more active than zein in this respect. The depressing effect was overcome by the addition of nicotinic acid or tryptophane to the ration. A dietary source of nicotinic acid was not apparently required if sufficient tryptophane was present in the ration.

The New Hampshire chick may require at least 5 mg of nicotinic acid per 100 gm of diet under certain extreme conditions.

By the addition of bone ossein to a highly purified ration, it was possible to obtain a nicotinic acid deficiency in laying hens with a resulting drop in egg production, body weight, and hatchability. By the same procedure a nicotinic acid deficiency was produced in young chickens receiving a practical ration high in corn and corn products.

A definite depression in growth rate and an increase in nicotinic acid deficiency symptoms were obtained by feeding certain combinations of arginine, glycine, and alanine. Thus, at least part of the depressing action of gelatin may be accounted for by its amino acid content.

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THE UTILIZATION OF IRON FROM DIFFERENT FOODS BY NORMAL YOUNG RATS[1]

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THREE FIGURES

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Most of the information concerning the utilization of the iron of different foods has been obtained by studies with animals rendered anemic by hemorrhage, exclusive milk feeding, or other means. In young rats made anemic by exclusive milk feeding, differences in the ability of different foods to regenerate hemoglobin have been observed. Most iron salts are readily utilized for hemoglobin regeneration, providing sufficient copper is present. Experiments by Rose and Vahlteich ('32) and Free and Bing ('36) have indicated that the iron of whole wheat is about as well utilized by the rat as that of ferric chloride, although there has been controversy on this point, apparently arising from a difference in the method of feeding employed (Nakamura and Mitchell, '43). The work of Rose and Kung ('32) and Rose, Vahlteich and MacLeod ('34) has shown that the iron of beef is not as well utilized by the rat as that of whole wheat; also that the iron of beef liver is more available than that of the muscle.

Ascham, Speirs and Maddox ('38) and Sheets and Ward ('40) demonstrated that the iron of leafy vegetables is less available for the rat than that of ferric chloride. That the

[1] The data in this paper were taken from a dissertation submitted by Orrea Florence Pye in partial fulfillment of the requirements for the degree of doctor of philosophy under the Joint Committee on Graduate Instruction, Columbia University.

iron of egg yolk is well utilized if there is an adequate supply of copper was indicated by the work of Rose, Vahlteich and MacLeod ('34) and Sherman, Hart and Elvehjem ('34).

Since so much of our information concerning the availability of iron from different foods has been acquired under the abnormal conditions of experimental anemia where factors not applicable to the normal situation might conceivably prove a complication, it seemed worthwhile to study the utilization of iron in normal young rats under dietary conditions approximating normal as closely as possible. The present investigation was designed, therefore, to follow the hemoglobin values of and determine the iron stored by normal young rats given, for a 6-week experimental period, supplies of iron which were within normal dietary limits. Since interest was centered on maintaining as nearly as possible a normal dietary situation, no attempt was made to compensate for the varying effect of different food supplements on the growth rate of the animals, but in order to counteract the effect of differences in body size, iron dosage was adjusted to body weight.

EXPERIMENTAL PROCEDURES

Two series of experimental diets were used. In Series I equal amounts of iron were supplied by different supplements, while in Series II differing levels of iron were fed.

Male albino rats 3 weeks of age were used, all from a stock colony reared on a diet of one-third whole milk powder, two-thirds whole wheat flour, and sodium chloride equal to 2% of the weight of the flour. Initial, weekly, and final hemoglobin determinations were made according to the Newcomer method on freely flowing blood obtained by cutting the tip of the tail of the animal. Several littermates were killed at 3 weeks of age to determine average iron content at the beginning of the 6-week experimental period. At the end of the period all animals were analyzed for iron content. All food materials were also analyzed. The method of analysis for iron was in general that employed by Rose and Hubbell ('38), Kunerth ('40), and Houghton ('42) in this laboratory. A method of

wet ashing with sulfuric and nitric acids suggested by Roberts, Beardsley and Taylor ('40) was adapted for use with a few of the foods analyzed.

Series I

Two basal rations were used. Basal diet A was one used successfully by Kunerth ('40) for rendering rats anemic. Since this diet with certain food supplements proved to be somewhat laxative, basal diet B (consisting of dried whole milk with daily administration of thiamine chloride and pyridoxine) was substituted in these cases. Eight food supplements (beef muscle, uncooked and cooked kale, uncooked and cooked spinach, whole wheat flour, beef liver, and egg yolk) and an iron salt (ferric chloride) were given in amounts to furnish the minimum quantity of iron needed by the young rat, 0.002 mg iron per gm of body weight daily (Rose and Hubbell, '38). Copper sulfate was added when the iron salt or egg yolk was given. The iron supplements were prepared at the beginning of the experimental period and all precautions taken to avoid accidental inclusion of iron in drying and pulverizing the foods. The beef muscle and beef liver were not cooked before drying but the egg yolk was. The animals were weighed weekly and the amount of iron required for each animal for the coming week computed according to this weight.

Series II

Four diets were used in Series II. Diet I consisted of one-third whole milk powder, two-thirds whole wheat flour, and sodium chloride equal to 2% of the total weight of the flour. Diet II was like Diet I except that half of the whole wheat flour was replaced by unenriched patent flour. To compensate for some of the losses in milling the flour, supplements were given as follows: 0.8 μg manganese and 0.3 μg copper per gm of rat per day, and 25 μg thiamine chloride, 2 μg riboflavin, and 4 μg pyridoxine per animal per day. Diet III had all of the whole wheat flour of Diet I replaced by unenriched

patent flour with mineral and vitamin supplements double those of Diet II. Diet IV was in all respects like Diet III except that sufficient ferric chloride was added to make the total iron content equal to that of Diet I.

RESULTS AND DISCUSSION

Series I

Average results are shown in table 1. The average weekly hemoglobin values of the rats receiving Diet A plus supplements are shown in figure 1. The average final hemoglobin level

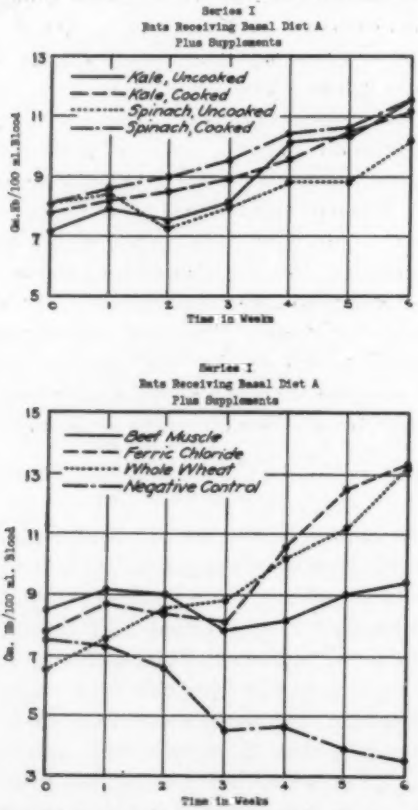

Fig. 1 Average weekly hemoglobin values of Series I rats receiving basal diet A plus supplements.

TABLE 1

Average results in 6-week experimental period. (Range of values given in parentheses.)

NO. OF ANIMALS	SUPPLEMENT OR DIET	WEIGHT		HEMOGLOBIN PER 100 ML OF BLOOD			IRON INTAKE		IRON CONTENT [1] PER GM OF RAT	RETENTION [1]
		Initial	Final	Initial	Final	Gain	From supplement	From basal diet		
		gm	*gm*	*gm*	*gm*	*gm*	*mg*	*mg*	*mg*	%
	Series I. Animals receiving a basal diet plus different supplements									
Diet A										
10	Beef muscle	39	184	8.5(7.1– 9.6)	9.4(8.4–10.5)	0.9	8.51	1.01	0.022(.015–.026)	32
5	Kale, uncooked	45	140	7.2(6.5– 8.0)	11.5(10.2–13.0)	4.3	6.82	1.05	0.023(.019–.028)	28
7	Kale, cooked	37	133	7.7(7.0– 8.2)	11.3(9.8–13.3)	3.6	6.61	1.03	0.024(.017–.027)	29
10	Spinach, uncooked	42	154	8.1(6.9–10.1)	10.1(8.7–12.1)	2.0	7.52	1.10	0.021(.016–.026)	26
7	Spinach, cooked	37	135	8.0(7.2– 8.8)	11.5(10.5–12.3)	3.5	6.54	1.08	0.026(.021–.033)	31
5	Whole wheat flour	40	140	6.5(6.2– 7.0)	13.3(12.8–13.9)	6.8	7.19	0.65	0.033(.029–.038)	42
12	Ferric chloride + copper sulfate	38	172	7.8(6.2– 8.9)	13.4(11.6–14.9)	5.6	8.18	1.42	0.031(.023–.036)	42
2	Negative control	40	103	7.4	3.4	—4.0		1.01	0.012	
Diet B										
6	Beef liver	38	193	9.1(8.5–10.2)	13.5(12.0–14.4)	4.4	8.98	1.36	0.029(.022–.038)	42
6	Egg yolk + copper sulfate	40	170	7.6(7.0– 8.2)	12.1(10.5–13.2)	4.5	8.31	1.27	0.025(.018–.030)	33
8	Ferric chloride + copper sulfate	37	152	10.0(9.3–10.5)	14.6(14.0–15.2)	4.6	7.27	1.35	0.033(.029–.039)	46
3	Negative control	39	115	9.3	3.8	—5.5		0.98	0.013	
	Series II. Animals receiving diets varying in iron content									
							Total from diet			
11	Diet I	37	165	7.8(6.2– 9.6)	14.7(14.0–15.3)	6.9	9.86		0.040(.030–.047)	54
12	Diet II	37	143	7.6(6.2– 8.9)	11.5(10.2–12.5)	3.9	5.60		0.026(.020–.031)	50
12	Diet III	39	134	7.4(6.2– 8.5)	6.6(5.1– 7.9)	—0.8	2.61		0.018(.014–.021)	46
12	Diet IV	39	166	8.2(6.7– 8.9)	13.9(11.8–15.1)	5.7	9.14		0.037(.029–.043)	49

[1] Based on net weight, that is, final live weight minus weight of intestinal tract.

attained by the animals receiving whole wheat flour (13.3 gm per 100 ml of blood) was practically identical with that reached by the rats given ferric chloride (13.4 gm). The rats receiving whole wheat flour made a greater gain in hemoglobin in the 6 weeks. This may have been due to the lower initial hemoglobin level in these rats, for in general, the animals with lower initial levels made greater hemoglobin gains in the 6-week period.

The iron content of the animals on whole wheat flour was very similar to that of the rats given ferric chloride, averaging 0.033 mg and 0.031 mg iron per gm of rat, respectively. In both cases the body iron was appreciably higher than in the 21-day-old animals, which averaged 0.022 mg iron per gm of body weight (range, 0.019–0.025 mg).

Of all the supplements fed, dried beef muscle was the least satisfactory in building hemoglobin, resulting in an average final level of 9.4 gm per 100 ml of blood, only slightly higher than the average initial level of 8.5 mg. The body iron per gm of rat of the animals fed the dried beef muscle did not increase in the 6-week period.

The average final hemoglobin values of the animals fed uncooked kale, cooked kale, uncooked spinach, and cooked spinach were, respectively, 11.5 gm, 11.3 gm, 10.1 gm, and 11.5 gm per 100 ml of blood. The average values for iron content were, in the same order, 0.023 mg, 0.024 mg, 0.021 mg, and 0.026 mg per gm of body weight. Ascham, Speirs and Maddox ('38) found slightly better hemoglobin response to spinach than to kale in rats rendered anemic. In this study the hemoglobin-building value of the iron of kale and spinach, cooked or uncooked, appears to be practically the same and in no case equal to that of the iron of whole wheat flour, ferric chloride, or beef liver.

The average weekly hemoglobin values of rats receiving Diet B plus supplements are shown in figure 2.

The animals fed dried beef liver attained an average final hemoglobin level of 13.5 gm per 100 ml of blood and an average iron content of 0.029 mg per gm, lower than the value of

0.033 mg per gm found in the rats given ferric chloride. The dried beef liver, as prepared in this study, while not as effective either in the production of hemoglobin or the deposit of iron in the tissues as the iron salt, still appeared to be well utilized by the normal young rat. In the experiments of Rose and Kung ('32) the iron of liver appeared to be effective in the regeneration of hemoglobin although not as effective as the iron of whole wheat. In this study the amount of iron given was adjusted to body weight. Therefore the rats

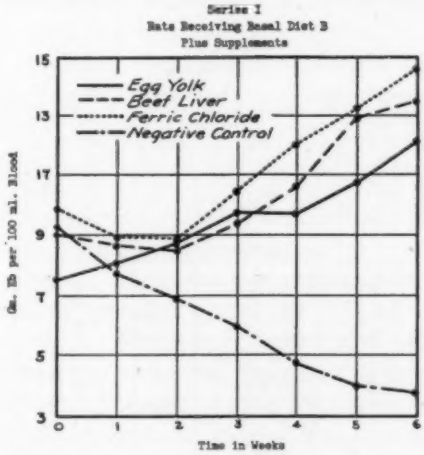

Fig. 2 Average weekly hemoglobin values of Series I rats receiving basal diet B plus supplements.

which were receiving the liver supplement and making greater weight gains received a larger supply of iron during the 6 weeks (10.34 mg) than the slower-growing animals on the iron salt supplement which obtained only 8.62 mg. If the iron ingested had been kept identical for both lots of animals, irrespective of weight, those on the liver supplement would have been at a disadvantage because they tended to grow faster.

The animals fed egg yolk plus 0.2 μg copper daily per gm of body weight attained a final level of only 12.1 gm hemo-

globin per 100 ml of blood. It may be that not enough additional copper was provided, as insoluble copper salts tend to form when egg yolk is fed. Copper may have been the limiting factor in hemoglobin formation but it could not bring about the low iron content per gm (0.025 mg) in these animals as it has been demonstrated that iron is stored in the absence of copper. Tompsett ('40) in an experiment in which mice were fed egg yolk as a source of iron found poor iron storage which he attributed to the interference of phosphatides and phosphoproteins with the absorption of iron.

Series II

Average results are shown in table 1 and average weekly hemoglobin values of the rats in this series in figure 3.

The iron content per 100 gm of the four diets was found upon analysis to be as follows: Diet I, 2.63 mg; Diet II,

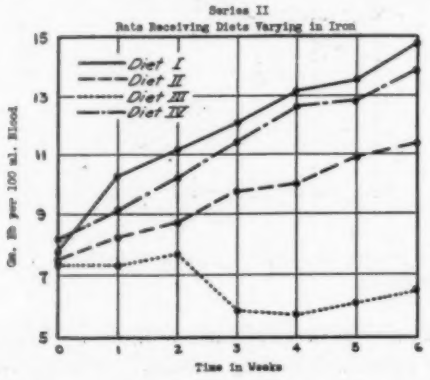

Fig. 3 Average weekly hemoglobin values of Series II rats receiving diets varying in iron content.

1.88 mg; Diet III, 0.97 mg; Diet IV, 2.54 mg. The average final hemoglobin levels per 100 ml of blood of the animals on the four diets were as follows: Diet I, 14.7 gm; Diet II, 11.5 gm; Diet III, 6.6 gm; Diet IV, 13.9 gm. Diet II did not provide sufficient iron to bring the hemoglobin up to the

normal level in the 6-week period. On Diet III the majority of the animals not only were unable to maintain the hemoglobin at the 21-day-old level, but showed a small decrease. On Diet I the hemoglobin values were all normal for 9-week-old rats. On Diet IV a few individual animals fell below the normal range.

Of the four diets, average iron content values per gm of rat were as follows: Diet I, 0.040 mg; Diet II, 0.026 mg; Diet III, 0.018 mg; Diet IV, 0.037 mg. Iron stores as well as hemoglobin values varied according to the quantity of iron ingested, although not in direct proportion.

Retention of iron. Percentage retention of iron on the different supplements and diets was computed according to the following formula: $\frac{\text{iron retained}}{\text{total iron ingested}} \times 100$. Iron retained was taken to be the difference between the average iron content (based on the final live weight of the animals minus the weight of the intestinal tract) at 3 weeks of age and after 6 weeks on the experimental diet.

Iron retention on Diet A plus supplements varied from 26% for uncooked spinach to 42% for both whole wheat flour and ferric chloride. When the percentage retention of the ferric chloride on the two basal diets is compared, Diet B has a slight, although probably not a significant advantage — 46% versus 42% on Diet A.

The percentage retention of iron was higher on the diets in Series II than on those in Series I. The percentage retention in Series II was as follows: Diet I, 54; Diet II, 50; Diet III, 46; and on Diet IV, 49. The highest retention in Series I was 46% on Diet B plus ferric chloride. The average level of iron intake on the diets in Series I was between that on Diets I and II in Series II. However, the animals on Diets I and II ingested a larger quantity of the total iron in the early part of the experimental period and a smaller quantity in the later part than the animals on the diets in Series I. Other variables, such as amino acids, copper, phytin, some of the vitamins, may also have affected the utilization of the iron in the different diets.

SUMMARY AND CONCLUSIONS

I. When normal young rats were fed daily 0.002 mg iron per gm of body weight from different sources and hemoglobin formation and iron retention in a 6-week period were used as the criteria of "availability," it was found that:

1. The iron of whole wheat flour was about equal in availability to that of ferric chloride.

2. The iron of beef liver was almost but not quite as available as that of ferric chloride.

3. The iron of the other food materials tested (beef muscle, egg yolk, uncooked and cooked kale, and uncooked and cooked spinach) was less available than that of beef liver; the differences among these supplements were small.

4. In general the order of "iron availability" of these different foods was the same as that observed in studies of hemoglobin regeneration in anemic rats, but the differences were smaller.

II. When normal young rats were given diets containing readily available sources of iron (I) whole wheat flour, (II) half whole wheat, half unenriched patent flour, (III) unenriched patent flour, (IV) unenriched patent flour plus ferric chloride, for a 6-week period, it was found that:

1. Hemoglobin production and iron storage were improved when the level of intake of iron averaged slightly more than 0.002 mg per gm of body weight daily (Diet I).

2. Levels of iron averaging lower than 0.002 mg iron per gm of body weight daily were insufficient to bring about good hemoglobin production; a level slightly lower resulted in a small hemoglobin gain (Diet II) but when the level of iron averaged below 0.001 mg (Diet III) no gain was observed in 6 weeks.

3. Levels of iron averaging lower than 0 002 mg iron per gm of body weight were insufficient to bring about much increase in the iron content per gm of rat in 6 weeks; a slightly lower level resulted in a slight increase (Diet II), but when the level of iron averaged below 0.001 mg (Diet III) a decrease was observed.

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The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the judges may recommend that it be given for important contributions over an extended period of time. The award may be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor.

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GENEVIEVE STEARNS,
College of Medicine,
State University of Iowa,
Iowa City, Iowa

CHAIRMAN, NOMINATING COMMITTEE

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Nominations are solicited for the 1947 Award of $1,000 established by Mead Johnson and Company to promote researches dealing with the B complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition and the formal presentation will be made at the annual meeting of the Institute, May 18–22, 1947.

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To be considered by the Committee of Judges, nominations for this award for work published in 1946 must be in the hands of the Chairman of the Nominating Committee by January 15, 1947. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

ROBERT R. SEALOCK,
Department of Chemistry,
Iowa State College,
Ames, Iowa

CHAIRMAN, NOMINATING COMMITTEE

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The rooster's legs are straight.

The boy's are not.

The rooster got plenty of vitamin D.

Fortunately, extreme cases of rickets such as the one above illustrated are comparatively rare nowadays, due to the widespread prophylactic use of vitamin D recommended by the medical profession.

One of the surest and easiest means of routinely administering vitamin D (and vitamin A) to children is MEAD'S OLEUM PERCOMORPHUM WITH OTHER FISH-LIVER OILS AND VIOSTEROL. Supplied in 10-cc. and 50-cc. bottles. Also supplied in bottles of 50 and 250 capsules. Council Accepted. All Mead Products Are Council Accepted.

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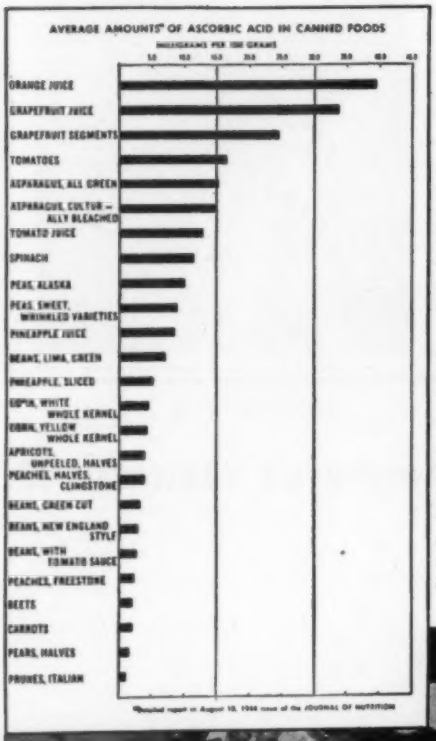
KNOWN FACTS

ABOUT THE NUTRITIVE VALUES OF FOODS PACKED IN CANS



Actual net values now available in chart form

Not just another chart, the chart on the left—and each chart in this series—presents *net* values, actual on-the-table values in foods packed in cans. Therefore, their significance is plain. As you know, the figures usually quoted in nutrition tables are *gross* figures for raw, *uncooked* foods which do not take into consideration losses which occur in transit from field to market, to kitchen, *and* in home preparation.

Quite true, canned foods are a truly great class of foods. However, in order for them to receive the public acceptance they merit, we realize that canned foods need the recommendation of leaders in the professional fields. We sincerely request your support. A series of twelve charts on the actual nutritional values of the most commonly consumed canned foods is now available in booklet form. For your copy, address: Can Manufacturers Institute, Inc., 60 East 42nd St., New York 17, N. Y.

Atlanta Constitution Photo—B. W. Calloway

HOW ROWENA THE COW GLAMORIZES MILK

The picture above shows a high dramatic point in a new smash-hit play, "Rowena the Skating Cow." This production has been staged several times by the first graders of Ed. S. Cook school in Atlanta, Georgia.

The imaginative story of Rowena—a cow who learns to skate, goes on the stage, but finally returns to her milk-producing duties—is only one of the many newsworthy projects Georgia schools are using to teach children better nutrition. For the past two years, a group of schools in and near Atlanta has pioneered in test projects of many kinds, and has had an excellent opportunity to measure the results.

The greatest fun, in work of this kind, is to see positive, statistical proof of progress. One group of more than 200 students in a Georgia school showed this change in milk-drinking habits: *In October, 1945 (before a special nutrition program), average daily consumption of milk per student was 1.30 cups. After 6 months of nutrition emphasis, consumption, while still below recommended intake, was upped by 61%—to 2.10 cups daily.*

To help elementary schools set up a nutrition education program, General Mills is working directly with test schools and teacher training colleges. As an outgrowth of this work a practical working plan for school, home, and community has been developed. This is implemented by booklets, posters, guidebooks, evaluation test forms and other needed materials. If you would like to know more about this plan and these materials, just mail the coupon below.

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Merck research has been directly responsible for many important contributions to the synthesis, development, and large-scale production of individual vitamin factors in pure form.

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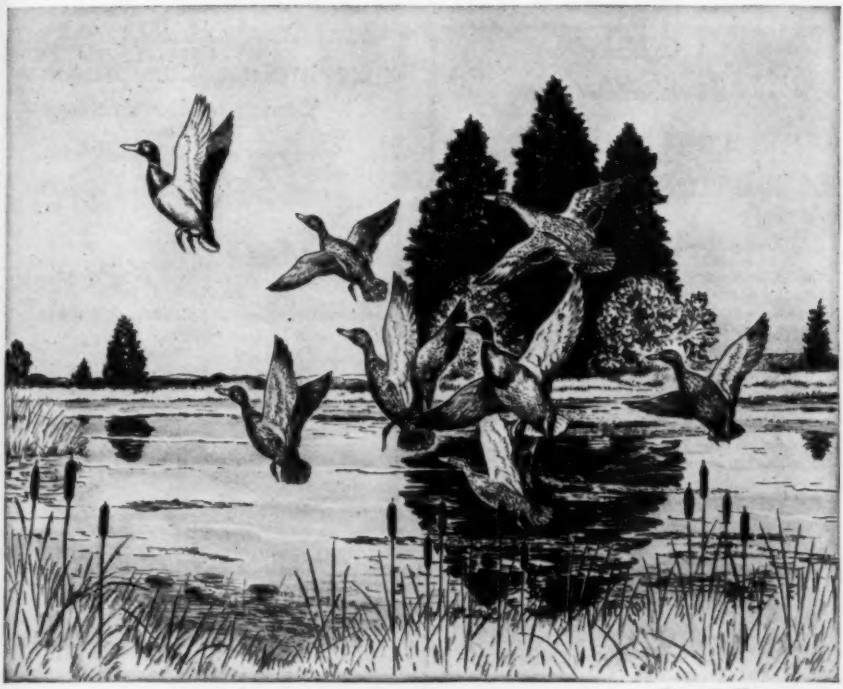

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Niacinamide—4 Mg.

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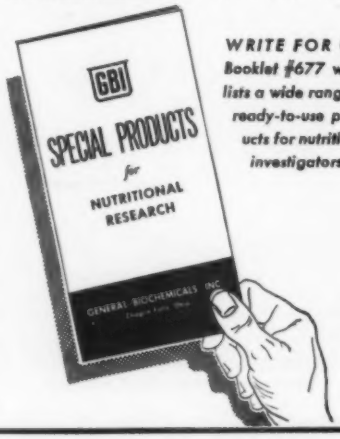

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Germ-Free Life Studies

LOBUND* REPORTS
(No. 1)

Editor: James A. Reyniers
Associate Editors:
Robert F. Ervin and Helmut A. Gordon

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Contents

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—James A. Reyniers, Philip C. Trexler and Robert F. Ervin

Germ-Free Life Applied to Nutrition Studies
—James A. Reyniers

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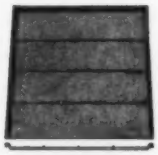

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Notes of interest from Borden's Quality Control Labs.

Subject:

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For centuries, cheese making has been an art carefully handed down from generation to generation.

At the same time, it has frequently been somewhat of a mystery, even to its most experienced practitioners. For, through the strange and not always predictable activities of molds and enzymes, the *same* cheese-making formula can not always be counted on to produce cheese with the *same subtle characteristics twice.*

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Some idea of the size of this task may be gained from the fact that, in normal times, Borden's handles as many as *forty different types* of cheeses, both foreign and domestic.

These cheeses range from the familiar 3- to 6-ounce package of American cream cheese, to the huge, 175-pound cartwheel of Swiss cheese. They include such widely differing types of cheeses as the North of Holland, red-colored Edam cannon ball . . . the long sausage-shape Italian Provolone ...the goat's milk Gjetost cheese of Norway.

CONCENTRATED NOURISHMENT

But what of cheese as a food? How does it rate in *nutritive value*? Here is a simple illustration. Approximately 4½ quarts of whole milk are required to produce one pound of American Cheddar Cheese. The cheese contains practically the same food essentials, some in altered proportions, found in the milk from which it is made.

Another way of looking at the nutritive value of cheese is this: Just **four ounces of cheese** contain the essential nutrients found in **three glasses of milk.** In other words, cheese is a **highly con-**

centrated form of nourishment in a highly palatable form.

Because cheese *is* such a *good* food, the Borden Company is constantly striving to bring to the American table even *finer* and *more nourishing* cheeses.

For cheese enthusiasts, an interesting folder, *"Principal Varieties of Domestic and Imported Natural Cheese,"* which gives pertinent information about different types of cheeses, is available upon request. For a copy, write to the Borden Company, 350 Madison Ave., New York 17, N. Y., Room 1605 C.

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INGREDIENTS	Grd. Whole Wheat, Alfalfa Leaf Meal, Soy Bean Oil Meal, Soy Bean Oil, Calcium Carbonate from Limestone, Bone Meal.	Grd. Whole Wheat, Alfalfa Leaf Meal, Soy Bean Oil Meal, Soy Bean Oil, Calcium Carbonate from Limestone, Salt (Iodized), Bone Meal, Ascorbic Acid, Irradiated Yeast.	Cane Molasses, Soy Bean Meal, Animal Liver Meal, Fish Meal, Meat Scraps, Condensed Buttermilk, Gluten Meal, Wheat Germ Oil, Oats, Wheat Bran, Wheat Flour Middlings, Yellow Corn, Hulled Barley, Hulled Oats, Wheat, Whole Milk Powder, Alfalfa Leaf Meal, A&D Feeding Oil, Steamed Bone Meal, Salt, Irradiated Yeast, Linseed Oil Meal, Corn Oil Meal, Calcium Carbonate.	Grd. Yellow Corn, Grd. Hulled Barley, Grd. Hulled Oats, Grd. Whole Wheat, Soy Bean Oil Meal, Meat Scraps, Whole Milk Powder not irradiated, Salt (not iodized), Precipitated Chalk, Cane Molasses, Alfalfa Leaf Meal.	Cane Molasses, Soy Bean Meal, Animal Liver Meal, Fish Meal, Meat Scraps, Condensed Buttermilk, Gluten Meal, Wheat Germ Oil, Oats, Wheat Bran, Wheat Flour Middlings, Yellow Corn, Hulled Barley, Hulled Oats, Wheat, Whole Milk Powder, Alfalfa Leaf Meal, A&D Feeding Oil, Steamed Bone Meal, Salt, Irradiated Yeast, Linseed Oil Meal, Corn Oil Meal, Calcium Carbonate.
AVERAGE ANALYSES (per cent)					
Protein	18.62	18.20	24.83	26.35	24.83
Fat	2.65	2.84	4.67	5.43	4.67
Fibre	13.70	12.80	4.86	4.15	4.83
Carbohydrates	61.66	61.52	49.32	46.99	49.32
Nitrogen Free X	47.96	48.27	44.46	42.84	44.46
Ash	6.56	7.64	9.53	11.68	9.53
MINERAL COMPOSITION by Chemical determination (per cent)					
Silica ($Si\ O_2$)	1.11	1.08	0.59	0.44	0.59
Chlorine (Cl)	0.16	0.71	0.96	1.50	0.96
Sodium (Na)	0.06	0.68	0.88	0.93	0.88
Potassium (K)	1.52	1.62	1.12	0.63	1.12
Calcium (Ca)	1.04	1.40	2.13	2.17	2.13
Phosphorus (P)	0.58	0.53	0.71	0.83	0.71
Sulphur (S)	0.21	0.21	0.24	0.23	0.24
Magnesium (Mg)	0.32	0.30	0.33	0.27	0.33
milligrams per 100 grams					
Manganese (Mn)	4.90	6.00	6.80	4.00	6.80
Iron (Fe)	36.00	44.00	37.00	33.60	37.00
Zinc (Zn)	2.60	2.50	2.00	2.40	2.00
Copper (Cu)	0.25	0.30	0.27	0.30	0.27
Boron (B)	2.45	2.18	1.37	1.23	1.37
Cobalt (Co)	.023	.019	.035	.018	.035
Iodine (I)	1.20	3.10	1.20	2.60	1.20
Fluorine (F)	Trace	1.50	1.90	.60	1.90
VITAMIN CONTENT by rat bioassay determination (per 100 gms)					
Vitamin A	1720-1880 Int'l. Units	1920-2160 Int'l. Units	610-680 Int'l. Units	210-300 Int'l. Units	610-680 Int'l. Units
Thiamine B_1	436-510 micrograms	410-452 micrograms	228-465 micrograms	215-370 micrograms	228-465 micrograms
Riboflavin B_2	424-722 micrograms	614-796 micrograms	538-652 micrograms	464-596 micrograms	538-652 micrograms
Pyridoxine B_6	486-617 micrograms	363-480 micrograms	209-275 micrograms	336-602 micrograms	209-275 micrograms
Pantothenic Acid	2760-3797 micrograms	2520-3244 micrograms	1989-2568 micrograms	2673-2984 micrograms	1989-2568 micrograms
Choline Equiv.	712-748 milligrams	896-998 milligrams	480-492 milligrams	384-462 milligrams	480-492 milligrams
†* Inositol (free)	36 milligrams	34 milligrams	61 milligrams	89 milligrams	61 milligrams
Vitamin D	21 U.S.P. Units	103 U.S.P. Units	148 U.S.P. Units	Inherent trace	148 U.S.P. Units
Alpha-Tocopherol	6 milligrams	8 milligrams	7 milligrams	3.2 milligrams	7 milligrams
† PABA	93 micrograms	73 micrograms	78 micrograms	43 micrograms	78 micrograms
by chemical determination (per 100 gms)					
Niacin	18.4 milligrams	18.6 milligrams	14.4 milligrams	16.00 milligrams	14.4 milligrams
Vitamin C	Inherent trace	fortified to 20. mgms.	3.60 milligrams	1.60 milligrams	3.60 milligrams
Carotene	1.46-2.91 milligrams	2.15-3.10 milligrams	.42-.45 milligrams	.24-.27 milligrams	.42-.45 milligrams
Choline	136 milligrams	156 milligrams	152 milligrams	148 milligrams	152 milligrams
RATIOS					
Ca : P	1.8:1	2:1	3:1	2.6:1	3:1
Ca : Mg	3.3:1	4.7:1	6.5:1	8:1	6.5:1
Na : K	1:25.	1:2.4	1:1.3	1:0.7	1:1.3
anti alopecia inositol diet level	Purified 80% / Stock 20%	Purified 85% / Stock 15%	Purified 70% / Stock 30%	Purified 72% / Stock 28%	Purified 70% / Stock 30%

* Mouse bioassay

† Also by microbiological assay

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